EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I ANTIGEN IN MOUSE NEURONS USING A RECOMBINANT HERPES SIMPLEX VIRUS

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ABSTRACT

The aim of the project was to determine whether MHC-Ia antigens can be expressed on the surfaces of HSV infected primary sensory neurons *in vivo*. To address this aim a recombinant HSV type 1 strain, called S-130, was used as a vector to introduce a mouse class I αC gene (H-2K^d), under independent transcriptional control, into primary sensory neurons *in vivo*. This novel approach ensured that the MHC-Ia antigen under investigation was expressed only in HSV infected neurons. S-130 synthesized H-2K^d transcripts and expressed surface H-2K^d antigens in a variety of cell lines, including a human neuroblastoma cell line (IMR-32), that expresses extremely low levels of MHC-Ia molecules constitutively. In K562 cells, H-2K^d was shown to be expressed at the cell-surface in association with β_2m. The S-130 H-2K^d expression cassette was efficiently transcribed in mouse sensory ganglia and significantly, H-2K^d antigens were expressed on the surfaces of S-130 infected mouse primary sensory neurons *in vivo*. Further, H-2K^d antigens were detected immunohistochemically in neurons latently infected with S-130.

Clearance of infectious virus and persistence of viral DNA in mouse ganglia was not significantly different for S-130 or its parental virus strain, C3b. In addition, S-130 and C3b reactivated from latently infected spinal ganglia with the same frequency. ICAM-1 was not detected on the surfaces of primary sensory neurons in uninfected mice, and only a small minority of neurons

expressed cell-surface ICAM-1 in response to HSV infection. In contrast, a significant proportion of glia cells in HSV infected ganglia were induced to express ICAM-1. Further work is required to determine whether failure to express ICAM-1 protects HSV infected neurons against CTL attack.

DECLARATION OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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1. INTRODUCTION AND REVIEW OF LITERATURE

1.1 The family Herpesviridae

1.1.1 Properties of herpesviruses

The family Herpesviridae is comprised of large, enveloped DNA viruses that infect a wide range of vertebrates. There are approximately 100 known herpesviruses, eight of which infect humans; herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), human herpesvirus 6 and 7 (HHV-6 and HHV-7) (Roizman, 1990) and Kaposi's sarcoma herpesvirus (KSHV) (Chang *et al.*, 1994; Cohen, 1994; Huang *et al.,* 1995; Su *et al.,* 1995; Schalling *et al.*, 1995). Herpesviruses are ubiquitous, highly successful pathogens which vary enormously in their biology and pathology and are capable of establishing lifelong latent infections in their host organisms (Clements, 1993).

Herpesviruses share a common virion structure. Viral particles are approximately 180-200nm in diameter and consist of a double stranded DNA core (Furlong *et al.,* 1972) packaged within a 100nm diameter icosahedral capsid, composed of 162 capsomeres (Wildy *et al.,* 1960; Horne & Wildy, 1961). The capsid is surrounded by an electron dense amorphorus material called the tegument (Morgan *et al.,* 1968), which in turn is contained within a

lipid envelope consisting of many surface glycoproteins (Morgan *et al.,* 1959; Epstein, 1962; Stannard *et al.,* 1987).

Herpesviruses share four distinct biological properties: (i) they encode a large number of enzymes involved in nucleic acid metabolism, (ii) viral DNA is synthesized and capsids are assembled in the nucleus, (iii) production of infectious progeny virus destroys infected cells *in vitro* and (iv) viral genomes persist for extended periods of time in a latent state in their natural hosts (Roizman, 1990).

1.1.2 Classification of herpesviruses

Herpesviruses have been classified into 3 subfamilies; alphaherpesvirinae, betaherpesvirinae and gammaherpesvirinae, based on biological properties such as (i) host range and tissue specificity, (ii) growth characteristics, (iii) cytopathology and (iv) characteristics of latent infection. Some herpesviruses have been classified into genera based on genome organization and DNA sequence homology (Roizman *et al.,* 1981).

1.1.2.1 Alphaherpesvirinae

Alphaherpesviruses are classified on the basis that they have a broad host range, short growth cycle, rapid spread in cell culture and cause cytocidal infections in a wide variety of cell and tissue types. They typically establish latent infections in sensory ganglia following productive infection in epithelial

cells (Wildy *et al.,* 1982), although some evidence suggests that virus may also persist in peripheral tissues (Scriba, 1977; Hill *et al.,* 1980; Openshaw, 1983; Cook *et al.,* 1987; Clements & Subak-Sharpe, 1988; Clements & Jamieson, 1989; O'Brien & Taylor, 1989; Pavan-Langston *et al.,* 1989; Claoue *et al.,* 1990). Alphaherpesviruses include the genera *Simplexvirus* (HSV-1, HSV-2, circopithcine herpesvirus 1, bovine mamillitis virus) and *Varicellovirus* (VZV, pseudorabies virus, equine herpesvirus type 1) (Clements, 1993).

1.1.2.2 Betaherpesvirinae

Betaherpesviruses are characterized by a narrow host range *in vitro* and *in vivo*, long growth cycle, slow virus spread and enlargement of infected cells (cytomegalia) (Wright, 1973; Roizman *et al.,* 1981). These viruses maintain latent infections in secretory gland epithelial cells, lymphoreticular cells, kidneys and other tissues (Clements, 1993). Unlike alphaherpesviruses, persistence in the nervous system has not been demonstrated. Betaherpesviruses have been divided into two genera; *Cytomegalovirus*, which includes HCMV, HHV-6 and HHV-7 (Salahuddin *et al.*, 1986; Frenkel *et al.*, 1990; Ho, 1991), and *Muromegalovirus*, which includes murine cytomegalovirus (MCMV). Persistent infections are usually asymptomatic in healthy adults, but disseminated infection and severe disease can occur in neonates and immunocompromised individuals (Honess, 1984).

1.1.2.3 Gammaherpesvirinae

Gammaherpesviruses have a host range restricted to the family or order to which the natural host belongs. All members of this subfamily infect and replicate in lymphoblastoid cells *in vitro* and some also cause lytic infection in certain epithelial and fibroblastic cells (Roizman *et al.,* 1981). Productive infection of T or B lymphocytes is often halted without production of progeny virus and latent infection can be established in lymphoid tissue (Craighead *et al.*, 1972; Roizman *et al.*, 1981). Gammaherpesviruses are divided into 2 subgroups; γ1 and γ2, on the basis of tropism for B or T lymphocytes, respectively (Deinhardt *et al.,* 1974; Frank *et al.,* 1976) and genetic complexity (Honess, 1984). The γ1 subgroup includes EBV and related viruses of Old World monkeys and apes which typically infect and immortalize B lymphocytes. EBV is associated with malignancies such as nasopharyngeal carcinoma and Burkitt's lymphoma (Miller, 1990). The γ2 subgroup includes herpesvirus saimiri and other viruses of New World monkeys and lower vertebrates. They usually persist as inapparent infections of T lymphocytes in the majority of adult members of their natural host species, although some can also cause lymphoproliferative diseases (Honess, 1984). The recently identified KSHV has been classified as a gammaherpesvirus on the basis of (i) association with Kaposi's sarcoma (KS) lesions, peripheral blood mononuclear cells and B lymphocytes of KS patients and B cell lymphomas (Chang *et al.,* 1994; Ambroziak *et al.,* 1995; Cesarman *et al.,* 1995) and (ii) it's sequence homology to EBV and herpesvirus siamiri (Chang *et al.,* 1994).

1.1.2.4 Other classification criteria

The initial classification of herpesviruses into subfamilies was based primarily on biological criteria but recently a genetic basis for classification has evolved as a wider range of herpesvirus DNA sequence data has become available. The genetic criteria available include (i) conservation of genes and the arrangement of gene clusters, (ii) the arrangement of terminal sequences involved with viral genome packaging and (iii) the distribution of nucleotides that are subject to methylation (Roizman, 1990).

Biological and genetic criteria for grouping herpesviruses into three subfamilies generally agree, with two notable exceptions: (i) On biological criteria Marek's disease herpesvirus (MDV) is a gammaherpesvirus, but by some genetic criteria it is an alphaherpesvirus (Buckmaster *et al.,* 1988), (ii) HHV-6 on the basis of biological criteria is classified as a gammaherpesvirus but genetically it is classified as a betaherpesvirus (Roizman, 1990).

The as yet unclassified channel catfish herpesvirus (icatlurid herpesvirus 1 [IHV1]) has recently been aligned most closely with betaherpesviruses, based upon distance measures and partial orderings of dinucleotide abundances (Karlin *et al.*, 1994). Genetic analyses based on genomic and sequence comparisons have also played an important role in elucidating evolutionary relationships among herpesviruses (McGeoch, 1989, 1990, 1992; Griffin, 1991; Bublot *et al.*, 1992; Karlin *et al.*, 1994).

1.2 Herpes simplex virus

Herpes simplex virus (HSV) infects millions of people worldwide. After recovery from productive infection, HSV establishes latency in sensory nerve ganglia. The ability of HSV to establish, maintain and reactivate from latency contributes greatly to it's impact on humans. Despite intensive study the molecular basis of latency is still poorly understood.

1.2.1 Clinical and epidemiological features of HSV infection

Humans are the only natural host for HSV. Infection is extremely common, irrespective of gender, geographical, seasonal or racial factors (Nahmias *et al.*, 1989). By the age of 40 years, up to 90% of the population is HSV seropositive (Nahmias & Roizman, 1973). During HSV infection long lasting neutralizing antibodies are produced and therefore seropositivity is indicative of exposure to HSV.

There are two serologically distinct types of HSV; HSV-1 and HSV-2 (Schneweis & Brandis, 1961). HSV-1 and HSV-2 are most commonly, but not exclusively, associated with oro-facial and genital infections, respectively (Dowdle *et al.*, 1967; Corey & Spear, 1986).

Primary infection with HSV-1 usually occurs in the mouth (gingivostomatitis) or eyes, causing vesicular lesions. Transmission is by direct contact. Genital

HSV-1 infection is also common; up to 40% of primary genital herpes is caused by HSV-1 (Barton *et al.*, 1982; Woolley & Kudesia, 1990; Cheong *et al.*, 1990). The prevalence of HSV-1 antibodies increases rapidly during childhood, from 50% at the age of 1 year to 60-85% at puberty (Nahmias *et al.*, 1970 a).

Primary infection with HSV-2 is typically associated with the genital region as a result of sexual contact with an infected individual and is usually acquired from puberty onwards (Kaufman *et al.*, 1973). The prevalence of HSV-2 seropositivity varies from 10% in higher socioeconomic groups to 20-60% in lower socioeconomic groups and is strongly related to past sexual activity (Nahmias *et al.*, 1970 a; Rawls *et al.*, 1972). Like primary oro-facial infections, primary genital infections are often asymptomatic (Rattray *et al.*, 1978; Adam *et al.*, 1979; Ekwo *et al.*, 1979; Corey, 1988) and it has been estimated that as few as 30% of infections are are clinically apparent (Mertz *et al.*, 1992).

HSV can sometimes cause fatal disease in a minority of individuals. HSV diseases of the central nervous system (CNS) usually cause devastating and frequently lethal neurological damage (Olson *et al.*, 1967; Whitley *et al.*, 1982; Corey & Spear, 1986). Such infections are the most common cause of sporadic fatal encephalitis (Peterslund, 1991). HSV induced encephalitis may result from primary or recurrent infection and immunocompromised individuals and

neonates are the highest risk groups (Pass *et al.,* 1979; Whitley *et al.,* 1984).

Neonatal HSV infection is usually caused by HSV-2 acquired from the mother by contact with genital lesions during childbirth (Nahmias *et al.,* 1967; Stagno & Whitley, 1985). However, acquisition can also occur postnatally through contact with family members with oral lesions (Nahmias *et al.,* 1967). Although mild infections restricted to the skin, eyes or mouth can occur, infection often disseminates, causing widespread damage to most organs and overall mortality is 65% (Hass, 1935; Hanshaw & Dudgeon, 1978; Peterslund, 1991).

Other HSV infections include keratoconjunctivitis (ocular infection), which is the most common cause of corneal blindness in the USA (Dawson & Togni, 1976), herpetic whitlow, (infection of the finger) (Corey *et al.,* 1983; Gill *et al.*, 1988) and herpes gladiatorium, which is found among wrestlers and is spread through skin abrasions (Selling & Kibrick, 1964; Wheeler & Cabraniss, 1965). In patients with skin disorders, including atopic dermatitis, HSV infection can cause large eruptions known as eczema herpeticum (Peterslund, 1991). HSV can also cause herpetic tracheobronchospasm in elderly immunocompetent individuals (Sherry *et al.*, 1988), herpetic oesophagitis and even hepatitis in immunosuppressed patients (Peterslund, 1991) and proctitis among homosexual men (Quinn *et al.*, 1981; Goodell *et al.*, 1983).

The high prevalence of HSV infection and the potential for virus reactivation from latency provides a large reservoir for transmission to susceptible individuals. In addition to clinically apparent disease, transmission via asymptomatic shedding represents an efficient and important mode of virus spread in both HSV-1 and HSV-2 infections (Buddingh *et al.*, 1953; Haynes *et al.*, 1968; Rattray *et al.*, 1978; Ekwo *et al.*, 1979; Hatherly *et al.*, 1980).

1.2.2 Components of the HSV virion

The HSV virion contains a linear, double stranded DNA genome of approximately 152 kilobase pairs (kb), with a G+C content of 68% (HSV-1) or 154 kb with a G+C content of 69% (HSV-2) (Becker *et al.,* 1968; Kieff *et al.,* 1971). The viral genome is packaged within an icosahedral capsid as bundles of parallel duplex strands (Booy *et al.*, 1991). The capsid is embedded in an amorphous mass of viral proteins called the tegument, which shows variable size and shape on electron microscopic examination (Roizman & Furlong, 1974).

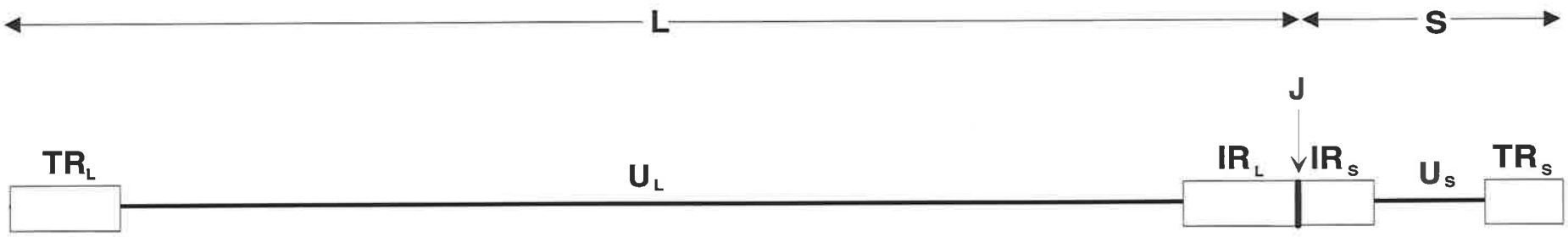
The complete nucleotide sequence of HSV-1 strain 17 has been determined. It encodes at least 73 unique proteins, of which 28 have unknown functions (McGeoch *et al.,* 1993). The HSV genome consists of two covalently linked segments, designated long (L, 126 kb) and short (S, 26 kb). Each segment contains unique sequences (U_L, 108 kb and U_S, 13 kb) which are each flanked by inverted repeat sequences located both internally (IR_L or *b'a'* and IR_S or

a'c') and terminally (TR_L or *ab* and TR_S or *ca*) (Sheldrick & Berthelot, 1975; Wadsworth *et al.,* 1975) (Figure 1.1). The *a* component of the repeat regions contains sequences which are highly conserved, but consists of a variable number of repeat elements (Wagner & Summers, 1978; Roizman, 1979 a, b; Davison & Wilkie, 1981). The L and S segments can freely invert relative to each other, generating 4 isomers which are found in equimolar amounts in HSV virion DNA preparations (Hayward *et al.,* 1975; Delius & Clements, 1976; Jacob *et al.,* 1979). The significance of segment inversion is unclear because the isomers appear to be functionally identical (Davison & Wilkie, 1983 a, b).

HSV virions contain at least 33 virally encoded proteins (Spear & Roizman, 1972; Heine *et al.,* 1974) and no detectable host proteins (Roizman & Sears, 1990). At least seven virion proteins are thought to be capsid components (Heine *et al.*, 1974; Marsden *et al.*, 1987). At present, 11 virally encoded glycoproteins (termed gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL and gM) have been identified (Ward & Roizman, 1994). All but 3 of these (gJ, gK and gL) are components of the virion envelope (Spear, 1993). On electron micrographs envelope glycoproteins appear as spikes projecting from the envelope (Stannard *et al.*, 1987). Virion glycoproteins appear to play an important role in mediating events that include attachment to, penetration of, and egress from target cells (Spear, 1993; Ward & Roizman, 1994).

Figure 1.1

Genome structure of HSV-1. The 152kb HSV genome consists of 2 covalently linked sequences; long (L) and short (S). The L segment is comprised of a unique region (U_L) flanked by the long terminal repeat (TR_L) and the long internal repeat (IR_L) regions. The S segment is comprised of a unique region (U_S) flanked by the short terminal repeat (TR_S) and the short internal repeat (IR_S) regions. The junction between the L and S segments of the genome is marked (J).



HSV-1 GENOME

MAP UNITS

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

0 20 40 60 80 100 120 140 150

KILOBASE PAIRS

The tegument contains no fewer than 11 identified proteins, most of which are phosphorylated (Haarr & Skulstad, 1994). The functions of several tegument proteins have been defined, including: (i) the virion host shut-off (VHS) protein, which shuts down host cell protein synthesis by destabilizing and degrading cellular mRNA (Nishioka & Silverstein, 1977, 1978; Read & Frenkel, 1983; Kwong *et al.,* 1988). (ii) Vmw65, also called α-transinducing factor (αTIF) or virion polypeptide 16 (VP16), which binds to the viral genome and cellular factors to form a potent transcription complex (αTIC) capable of inducing transcription of many viral genes (Batterson & Roizman, 1983; Campbell *et al.,* 1984; Pellet *et al.,* 1985; Marsden *et al.,* 1987; Gerster & Roeder, 1988; O'Hare *et al.,* 1988; Preston *et al.,* 1988; Spector *et al.,* 1991).

1.2.3 Replication of HSV

In permissive cells *in vitro,* HSV replication can be divided into four stages: (i) attachment and penetration, (ii) viral gene expression, (iii) viral DNA replication and (iv) virion assembly and egress.

1.2.3.1 Attachment and penetration

Attachment of the virion envelope to a susceptible cell initiates the replicative cycle of HSV. The principle component of the initial interaction of the virion with the cell is binding of gC to cell-surface heparan sulphate (Wudunn & Spear, 1989; Campadelli-Fiume *et al.,* 1990; Shieh *et al.,* 1992; Gruenheid *et*

al., 1993). An alternative attachment mechanism has been proposed because gC negative HSV mutants can still infect cells *in vitro*, albeit at reduced efficiency (Herold *et al.,* 1991). In this respect, Lee and Fuller (1993) demonstrated that HSV binds not only to a heparin-like cell component, but also a different cell-surface moiety that is also bound by gD and possibly gH. gB can also bind heparan sulphate but is dispensable for virus attachment (Cai *et al.,* 1988; Herold *et al.,* 1991; Herold *et al.*, 1995).

Studies on cell-specific kinetics and the efficiency of virus entry into susceptible cell lines has demonstrated two distinct phases of attachment that occur before penetration; an initial phase that is sensitive to removal by heparin-containing buffers, and a second phase that is heparin resistant. The latter seems to be the rate limiting event in viral entry (McClain & Fuller, 1994). Although HSV can enter cells by endocytosis, this results in a non-productive infection due to virus degradation in lysosomes (Wittels & Spear, 1991; Campadelli-Fiume *et al.,* 1988).

Attachment to the cell-surface activates the penetration process. Penetration is also mediated by viral glycoproteins and results in fusion of the viral envelope to the cell plasma membrane with subsequent release of the nucleocapsid directly into the cytoplasm (Morgan *et al.,* 1968; Para *et al.,* 1980; Johnson *et al.,* 1984; Fuller & Spear, 1987; Fuller *et al.,* 1989).

Penetration is thought to be a multistep process and studies utilizing mutant viruses and neutralizing antibodies have shown that it requires at least three glycoproteins; gB (Manservigi *et al.,* 1977; Sarmiento *et al.,* 1979; DeLuca *et al.,* 1982; Highlander *et al.,* 1988; Cai *et al.,* 1988), gD (Fuller & Spear, 1987; Highlander *et al.,* 1987; Johnson & Ligas, 1988; Ligas & Johnson, 1988) and gH (Little *et al.,* 1981; Gompels & Minson, 1986; Desai *et al.,* 1988; Fuller *et al.,* 1989; Forrester *et al.,* 1992). In the absence of these glycoproteins, virus binds to cells with normal efficiency, but does not penetrate. Viral glycoproteins gK and gL have also been implicated in playing a role in HSV penetration (Debroy *et al.,* 1985; Hutchinson *et al.,* 1992). Fuller and Lee (1992) proposed a model of virus entry involving a series of interactions between the virion envelope and the cell plasma membrane that trigger virion disassembly, membrane fusion and capsid penetration. In this model, gD stablizes attachment prior to penetration, and gH participates in the process of membrane fusion.

Once a virion penetrates the cell membrane, the uncoated viral capsid and tegument proteins are transported to nuclear pores, probably via the cellular cytoskeleton. Subsequently, viral DNA is released into the nucleoplasm (Tognon *et al.,* 1981; Batterson *et al.,* 1983; Kristensson *et al.,* 1986). Some tegument proteins, including Vmw65 and VHS, also enter the nucleus. Immediately after entry of viral DNA into the nucleus, linear virion DNA is thought to circularize (Poffenberger & Roizman, 1985). Circularization occurs

before transcription of viral genes.

1.2.3.2 Viral gene expression

Transcription of viral genes is dependent on host cell RNA polymerase II (Costanzo *et al.,* 1977). Transcription occurs in a co-ordinately regulated and sequentially ordered fashion (Honess & Roizman, 1973, 1974). Based on kinetics and the requirements for their expression, HSV viral genes have been divided into 3 temporally regulated groups; (i) α or immediate early (IE) genes, (ii) β or early (E) genes and (iii) γ or late (L) genes.

The α genes are the first to be expressed and their transcription does not require prior *de novo* synthesis of viral peptides (Honess & Roizman, 1974; Post *et al.,* 1981). There are five α proteins, designated infected cell polypeptide (ICP) 0, 4, 22, 27 and 47. A feature of all α genes is the presence of an upstream *cis*-acting element (Mackem & Roizman, 1982) containing the motif TAATGARAT (where R is a purine residue). All α genes are upregulated by an α-transinducing complex (αTIC), comprising of αTIF (Batterson *et al.*, 1983; Campbell *et al.*, 1984; Pellett *et al.*, 1985; Weinheimer *et al.*, 1992), a cellular transcription factor (Oct-1) (O'Hare & Goding, 1988; O'Hare *et al.*, 1988; Stern *et al.*, 1989) and at least one other cellular component (CFF) (Gerster & Roeder, 1988; Xiao & Capone, 1990; Katan *et al.*, 1990). αTIC binds to the TAATGARAT element and this interaction is essential for α gene activation (Gaffney *et al.*, 1985; McKnight *et al.*, 1987).

With the exception of ICP47, the α proteins regulate α, β and γ gene expression (Preston, 1979 a, b; DeLuca & Schaffer, 1985; Stow & Stow, 1986; Sacks & Schaffer, 1987; Roberts *et al.,* 1988; Cai & Schaffer, 1989; Everett, 1989; McCarthy *et al.*, 1989; Smith *et al.,* 1992). However, only ICP4 and ICP27 are essential for viral replication *in vitro* (Watson & Clements, 1978; Preston, 1979 a, b; Sacks *et al.,* 1985). The functions of ICP22 and ICP47 are not fully understood. Neither are essential for viral replication (Post *et al.,* 1981; Mavromara-Nazos *et al.,* 1986), however ICP22 null mutants show restricted host range and appear to play a role in enhancing some γ genes (Sears *et al.,* 1985 a).

Synthesis of α polypeptides peaks 2-4 hours after infection, but α proteins continue to accumulate late into infection (Honess & Roizman, 1974). Immediate early gene products may act as targets for cytotoxic T lymphocytes (CTLs) (Martin *et al.,* 1988) and also for natural killer (NK) cell mediated lysis (Fitzgerald-Bocarsly *et al.,* 1991).

The β genes are synthesized at a peak rate 5-7 hours after infection and require the presence of functional α proteins for their expression (Honess & Roizman, 1974, 1975). β gene products make up most of the non-structural proteins and are almost exclusively involved in DNA replication and nucleotide metabolism (Wu *et al.*, 1988). Synthesis of β proteins signals the onset of viral DNA replication (Honess & Roizman, 1974). β proteins comprise 2 groups; β_1 and

β_2. β_1 proteins are expressed very early after infection and require ICP4 for their synthesis (Honess & Roizman, 1974, 1975; Roizman & Sears, 1990). Expression of β_2 proteins is delayed and follows the expression of β_1 proteins. There are 7 β gene products that are essential for HSV DNA replication including the replication origin binding protein (UL9 gene), DNA polymerase (UL30 gene) and its associated catalytic factor (UL42 gene), single stranded DNA binding protein (termed ICP8; UL29 gene) and proteins of the helicase/primase complex (UL5, UL8, UL52 genes) (Challberg, 1986; Wu *et al.,* 1988). A further 5 β proteins including thymidine kinase (TK; UL23 gene) and ribonucleotide reductase (a dimer comprised of UL39 and UL40 gene products) are involved in nucleotide metabolism.

γ gene products are detectable approximately 9 hours after infection. They are mainly structural virion proteins required for capsid assembly and DNA packaging (Honess & Roizman, 1974). Like β proteins, γ proteins are subdivided into 2 groups; γ_1 and γ_2 (Roizman & Sears, 1990). γ_1 protein expression is induced by, but not absolutely dependent on viral DNA synthesis (eg. αTIF and glycoproteins gB and gD). γ_2 protein expression stringently requires prior viral DNA synthesis (eg. glycoproteins gC and gE) (Conley *et al.,* 1981; Silver & Roizman, 1985; Mavromara-Nazos & Roizman, 1987; Roizman & Sears, 1990). γ genes are transactivated and transrepressed by α and β gene products including ICP4, ICP0, ICP22, ICP27 and ICP8 (Costa *et al.*, 1985; Everett, 1986; Michael *et al.*, 1988; Sekulovich *et al.*, 1988; Gao &

Knipe, 1991).

1.2.3.3 Viral DNA replication

In permissive cells *in vitro*, replication of viral DNA starts approximately 3 hours after infection and continues for 9-12 hours (Roizman & Roane, 1964; Roizman *et al.,* 1965; Roizman & Sears, 1990).

The HSV genome contains 3 origins of replication. Two of these, $oriS_1$ and $oriS_2$ map within the repeated sequences of the S component (Mocarski & Roizman, 1982 a; Stow, 1982; Stow & McMonagle, 1983; Deb & Doelberg, 1988) and the other, oriL, maps within U_L (Weller *et al.,* 1985). Deletion studies have shown that one copy of any ori sequence may be sufficient for viral DNA replication (Igarashi *et al.,* 1993).

Restriction endonuclease analysis and sedimentation studies suggest that HSV DNA replicative intermediates are high molecular weight concatemeric molecules, in which the termini are joined together in a head to tail arrangement (Ben-Porat & Tokazewski, 1977; Jacob & Roizman, 1977; Jacob *et al.,* 1979; Jongeneel & Bachenheimer, 1981). It has therefore been proposed that HSV replicates by a "rolling circle" mechanism, using circular HSV genomes as templates (Ben-Porat & Tokazewski, 1977; Becker *et al.,* 1978; Jacob *et al.,* 1979; Roizman, 1979 a; Poffenberger & Roizman, 1985; Garber *et al.,* 1993). Unit length HSV genomes are thought to be cleaved from newly

synthesized concatemeric DNA (Jacob *et al.,* 1979; Vlazny *et al.,* 1982). Cleavage of concatemers is linked to the packaging of progeny genomes into preformed empty capsids and is dependent on the *a* sequence (Vlazny *et al.,* 1982; Stow & McMonagle, 1983; Varmuza & Smiley, 1985; Deiss *et al.,* 1986; Deiss & Frenkel, 1986; Smiley *et al.,* 1992).

The generation of 4 isomeric forms of HSV DNA by L and S segment inversion is associated with the process of DNA replication, cleavage and packaging (Hayward *et al.*, 1975; Delius & Clements, 1976). The molecular basis of this segment inversion is not fully understood. Two isomers can form from a single monomeric template by alternate cleavage during the maturation and packaging of concatemeric or circular DNA. The remaining two isomers must be generated by homologous recombination and the repeated *a* sequence appears to play a role in this process (Mocarski *et al.,* 1980; Smiley *et al.,* 1981; Mocarski & Roizman, 1982 b; Bruckner *et al.,* 1992; Dutch *et al.,* 1992).

It has recently been shown that replicative intermediates consist of head to tail concatemers in which the orientation of the L segment of adjacent viral genomes is frequently opposed (P/I concatemers) (Zhang *et al.*, 1994; Bataille & Epstein, 1994; Severini *et al.*, 1994). From these structures it is possible to generate all 4 isomers using alternate cleavage sites. P/I concatemers were detected at an early stage of DNA synthesis when replicating intermediates

appeared to be in a circular configuration. These observations cannot be explained by a conventional rolling circle replication model. Thus, Zhang *et al.* (1994) suggested that HSV may replicate in a manner similar to that of the 2μ plasmid of yeast (Futcher, 1986). This model involves both bidirectional initiation of DNA synthesis and intragenomic recombination events during DNA replication.

1.2.3.4 Virion assembly and egress

Capsids are assembled in the nucleus (Ben-Porat & Kaplan, 1973) and newly synthesized viral DNA is inserted into preformed empty capsids (Deiss *et al.*, 1986; Deiss & Frenkel, 1986). The major internal protein constituent of the capsid; ICP35, and the UL33 gene product, play an important role in the assembly of full capsids (Newcomb & Brown, 1991; al-Kobaisi *et al.*, 1991). Late in infection, the appearance of thick patches in the nuclear membrane signals the initiation of capsid release from the nucleus (Nii *et al.*, 1968). Nucleocapsids attach to patches of modified inner lamellae on the nuclear membrane, then bud into the perinuclear space, resulting in envelopment of the capsid (Darlington & Moss, 1969; Morgan *et al.*, 1968; Nii *et al.*, 1968). Vlanzy *et al.* (1982) showed that envelopment is dependent on the presence of a full length HSV genome in the capsid. Enveloped virions transit through the cytoplasm via the golgi and are finally released from the cell by exocytosis (Johnson & Spear, 1982). The complete replicative cycle of HSV in cultured cells takes 18-20 hours and results in cell death (Darlington & Granoff, 1973).

1.3 Pathogenesis of HSV infection

The pathogenesis of herpes simplex has been extensively studied both in animal models and humans. HSV pathogenesis can be divided into 3 distinct stages: (i) primary infection, which typically involves viral replication at the site of infection and access to sensory nerve endings, (ii) latent infection, in which the viral genome is sequestered in a non-replicating state in primary sensory neurons and (iii) recurrent infection, when viral genomes reactivate from the latent state and generate new infectious virus particles.

1.3.1 Primary infection

HSV is transmitted by direct contact and the initial phase of HSV infection in a susceptible individual involves the exposure of virus to mucosal surfaces or abraded skin, which permits virus entry. Virus replicates in epidermal cells, resulting in cell death. A clinically apparent vesicular lesion may develop (Darlington & Granoff, 1973) but primary infection is often asymptomatic. Multinucleated giant cells are a characteristic of infection and are found in vesicular fluid, together with large amounts of infectious virus, inflammatory cells and cellular debris (Darlington & Granoff, 1973; Rawls, 1985). Following development of a lesion, the vesicular fluid dries out and the lesion crusts, before healing with occasional residual scarring (Whitley, 1990; Timbury, 1993). During the inflammatory process, draining lymph nodes often become enlarged due to virus spread from the infected epithelium (Whitley, 1990).

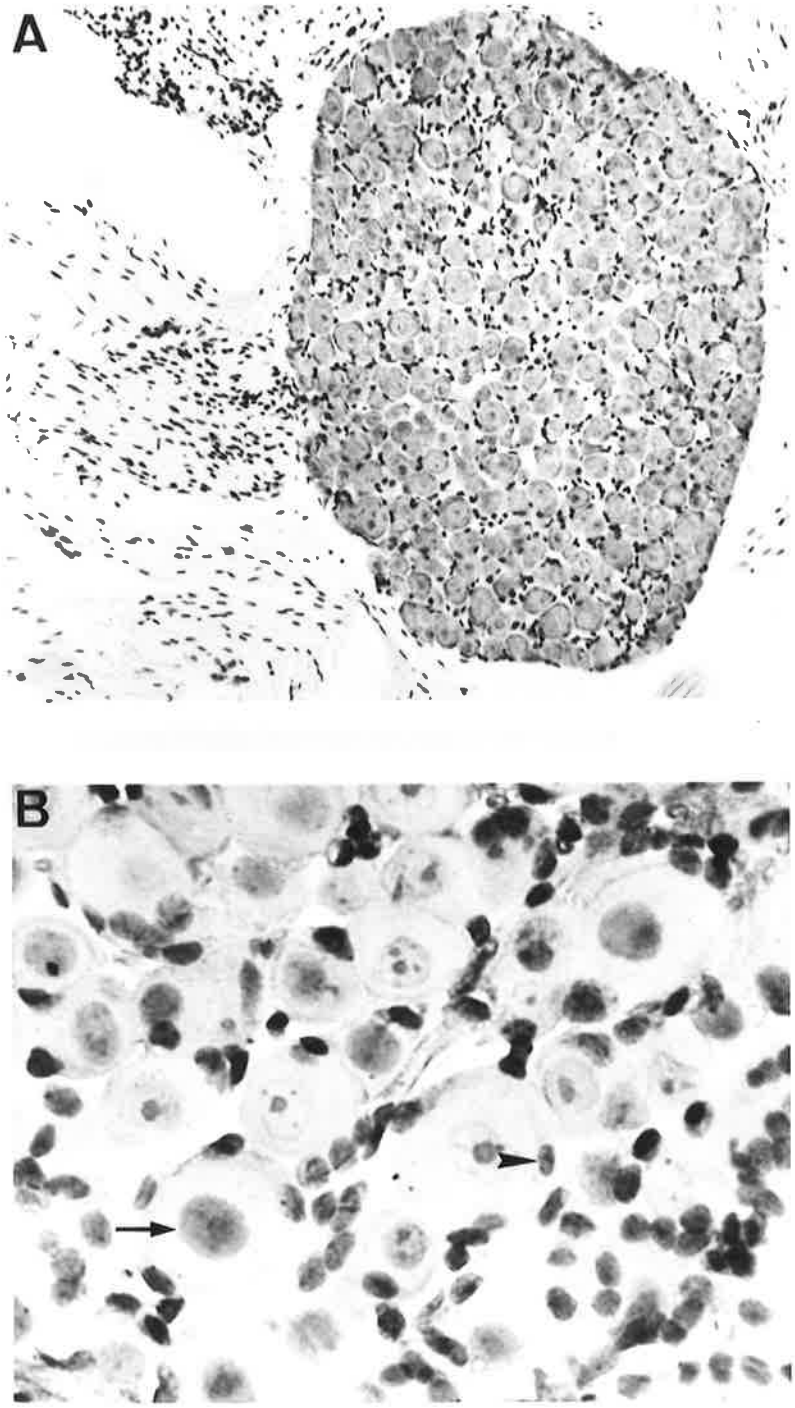
Sensory dorsal root ganglia contain large primary sensory neurons which are tightly encapsidated by satellite cells (Warwick & Williams, 1973) (Figure 1.2). The axons of primary sensory neurons bifurcate at a T-junction sending one branch into the CNS via the nerve root and another to the periphery along the spinal nerve. Schwann cells surround many axons in the spinal nerve. The sensory nerve fibres from a single dorsal root ganglion innervate a segment of skin called a dermatome (Foerster, 1933). During primary peripheral infection, HSV particles enter sensory nerve endings at the infected site and travel by retrograde axonal flow to neuronal cell bodies in sensory ganglia (Kristensson *et al.*, 1971; Cook & Stevens, 1973; Baringer & Swoveland, 1973).

Virus replicates in neurons and infectious virus can be recovered from ganglionic homogenates (Rock, 1993). Electron microscopic studies suggest that infection within satellite cells is abortive (Dillard *et al.,* 1972; Cook & Stevens, 1973). Virus particles can be detected in occasional Schwann cells (Rabin *et al.,* 1968; Severin & White, 1968). During productive infection, virus can reseed the site of primary infection (Cook & Stevens, 1973). After several days productive infection is terminated by cell-mediated immune responses (Nash & Cambouropoulus, 1993).

Figure 1.2

(A) Photomicrograph (magnification x 75) of a section through a C3H/HEJ mouse spinal ganglion, showing neurons (large rounded cells), satellite cells (small flattened cells surrounding neurons) and nerve fibres (centre left).

(B) Photomicrograph (magnification x 384) of a section through a C3H/HEJ mouse spinal ganglion. The nucleus of a neuron (arrow) and satellite cell (arrowhead) are indicated.


A
B

1.3.2 Latent infection

The establishment of a life-long latent infection is a fundamental feature of HSV-1 and HSV-2 infections. A central feature of latency is that the viral genome is sequestered in a non-replicating state and no viral proteins or infectious virus can be detected during this period (Stevens, 1989). The dorsal root ganglion has long been thought to be the site of latent HSV, based on histological changes in ganglia innervating sites of HSV recurrence (Goodpasture, 1929). Direct evidence supporting this hypothesis was provided by studies which showed that following explant cultivation, infectious virus could be recovered from sensory ganglia of latently infected animals (Stevens & Cook, 1971; Stevens *et al.,* 1972; Cook & Stevens, 1973) and from trigeminal ganglia of human cadavers (Baringer & Swoveland, 1973; Bastian *et al.,* 1972; Plummer, 1973; Warren *et al.,* 1977; Londsdale *et al.,* 1979). The viral genome has also been detected by Southern blotting in DNA extracted from latently infected ganglia (Rock & Fraser, 1983, 1985; Efstathiou *et al.*, 1986; Simmons *et al.*, 1992 a; Slobedman *et al.*, 1994). Further studies have demonstrated that latent HSV is sequestered in sensory neurons: (i) HSV can be reactivated from latently infected mouse or rabbit ganglia but not from adjacent nerves or nerve roots (Baringer & Swoveland, 1973; Cook *et al.,* 1974; Walz *et al.,* 1974). (ii) During HSV reactivation *in vivo* and *in vitro* viral antigens are detected first in neurons (Cook *et al.,* 1974; McLennan & Darby, 1980; Kennedy *et al.*, 1983; Wroblewska *et al.*, 1989). (iii) During latency, viral transcripts can be detected in neuronal nuclei (Galloway *et al.,*

1979, 1982; Tenser *et al.,* 1982; Stroop *et al.,* 1984; Stevens *et al.,* 1987).

Latent HSV has been isolated from tissues other than sensory ganglia including autonomic ganglia, such as the superior cervical and vagal ganglia (Price *et al.,* 1975; Martin *et al.,* 1977; Warren *et al.,* 1978), the adrenal medulla (Cook & Stevens, 1976) and brains of latently infected animals (Knotts *et al.,* 1973; Cook & Stevens, 1976; Cabrera *et al.,* 1980; Rock & Fraser, 1983). Peripheral tissues such as the skin and mucous membranes have also been implicated as sites of latency in humans and animal models of HSV infection (Scriba, 1977, 1981; Hill *et al.,* 1980; Schimeld *et al.,* 1982; Subak-Sharpe *et al.,* 1984; Clements & Subak-Sharpe, 1988; Clements & Jamieson, 1989; O'Brien & Taylor, 1989; Pavan-Langston *et al.,* 1989). Nonetheless, there is overwhelming evidence that the nervous system, specifically sensory ganglionic neurons, are the predominant site of latency.

1.3.3 Recurrent infection

The ability of HSV to reactivate from the latent state is an essential step for the spread of HSV in the population. Reactivation from latency may result in asymptomatic viral shedding (recurrence) or clinically evident disease (recrudescence) (Wildy *et al.,* 1982). Although the mechanisms underlying virus reactivation are poorly understood, recurrent infection may occur spontaneously or in response to provocative stimuli such as skin trauma, sun-burn, ultraviolet light or stress (Ship *et al.,* 1967; Nahmias & Roizman, 1973).

Whatever the processes involved, following reactivation newly made virions travel from the sensory ganglia to muco-cutaneous sites (Roizman, 1966). Using an *in vitro* model consisting of human fetal dorsal root ganglia, Penfold *et al.* (1994) demonstrated a specialized mode of virus transport, assembly and egress in sensory neurons, involving microtubule-associated anterograde axonal transport of unenveloped nucleocapsids, with separate transport of glycoproteins to the distal regions of the axon. Virion assembly occurred at the axon terminus prior to virus emergence.

Recurrent HSV infection occurs almost exclusively in individuals that possess neutralizing antibodies to the homologous virus, suggesting that it is due to reactivation of latent endogenous virus (Andrews & Carmichael, 1930; Burnet & Williams, 1939). Although exogenous re-infection has been reported (Nahmias *et al.,* 1970 b; Buchman *et al.,* 1979; Heller *et al.,* 1982), it's very low frequency does not account for the vast majority of apparent reactivations (Schmidt *et al.,* 1984; Lakeman *et al.,* 1986).

The immune response plays an important role in the control of recrudescence because immunosuppression increases the severity of lesions. However, recurrent infection occurs in the presence of an established immune response to the virus. It appears that effective immune recognition only occurs after the process of reactivation is considerably advanced (ie. after lesions have begun to develop) (Simmons & Nash, 1984).

Although HSV efficiently kills epidermal cells and permissive cells in culture, whether neurons *in vivo* are destroyed during the process of reactivation remains unclear. Surgically induced reactivation in mice causes a reduction in the number of latently infected sites, implying that neurons are killed during reactivation of HSV by the ensuing productive infection (McLennan & Darby, 1980). However, the survival of some neurons is supported by (i) the necessity for virions to be transported from the neuronal cell body to the periphery via an intact axonal transport process and (ii), the observation that some individuals suffer hundreds of recrudescences within a single dermatome with little or no sensory loss (Simmons *et al.,* 1992 b).

1.4 Molecular aspects of HSV latency

1.4.1 Gene expression and the establishment of latency

Determination of the genetic requirements for the establishment of latency has been complicated by the presence of a concurrent productive infection. A prominent approach to the study of establishment of latency has been the construction and characterization of viral mutants that are defective at various stages of the replicative cycle. The ability of these viruses to establish latency has been assessed by explant reactivation, detection of latency associated transcripts (LATs; see section 1.4.3) and the detection of HSV DNA sequences.

Initial studies utilized temperature sensitive (*ts*) HSV mutants that where non-permissive for viral replication at the internal temperature of mice (38.5°C). These studies suggested that viral replication was not required for the establishment of latency because *ts* mutants were able to establish a latent infection that could be reactivated by explant culture at permissive temperatures (31-33°C) (Lofgren *et al.,* 1977; McLennan & Darby, 1980; Watson *et al.,* 1980; Al-Saadi *et al.,* 1983).

Further studies have utilized mutant HSVs with specifically defined mutations.

Thymidine kinase (TK) deficient mutant HSVs can replicate effectively in cells *in vitro* (Dubbs & Kit, 1964) and in peripheral tissues *in vivo* (Tenser *et al.*, 1979, 1981), but cannot replicate in or be reactivated from non-dividing cells such as neurons (Field & Wildy, 1978; Tenser & Dunstan, 1979; Tenser *et al.*, 1979; Price & Kahn, 1981), which contain low levels of cellular TK (Jamieson & Subak-Sharpe, 1974; Tenser & Dunstan, 1979). However, numerous studies have shown by a variety of techniques, including the detection of LATs and viral DNA and recovery of TK deficient HSV by superinfection with wild-type HSV, that TK deficient HSVs can establish latent infection (Sears *et al.*, 1985 b; Ho & Mocarski, 1988; Efstathiou *et al.*, 1989; Coen *et al.*, 1989; Leist *et al.*, 1989 a; Tenser *et al.*, 1989; Kosz-Vnenchak *et al.*, 1990; Katz *et al.*, 1990; Jacobson *et al.*, 1993; Slobedman *et al.*, 1994). Therefore, viral TK is dispensable for the establishment of latency, but appears to play an important

role during viral reactivation by facilitating replication in neurons.

Viruses with mutations in α genes, which include those essential for transactivation of other HSV genes, have also been assessed for their ability to establish a latent infection. Deletion mutants of α22 (Sears *et al.*, 1985 a; Meignier *et al.*, 1988), α47 (Meignier *et al.*, 1988) and α0 (Clements & Stow, 1989) are capable of establishing latency and can be reactivated by explant culture. In addition, HSV DNA and LATs have been detected in ganglia of mice latently infected with α4 deletion mutants (Katz *et al.*, 1990; Sedarati *et al.*, 1993). Further, α4 deficient mutants can be reactivated by superinfection with an α4 competent agent (Sedarati *et al.*, 1993). These studies demonstrated that genes associated with the first stages of productive infection are not essential for the establishment and maintenance of latency.

Repression of α genes by inactivation of the αTIF may represent the crucial determinant for the establishment of latency. Ace *et al.* (1989) constructed a HSV-1 mutant (*in*1814) which synthesized a specifically altered αTIF protein that still fulfilled its structural role but was deficient in α gene transactivation. Studies using this virus showed that although replication in mice during productive infection was either drastically reduced or undetectable, latency was established at wild-type levels and virus could be reactivated by explant culture (Steiner *et al.*, 1990; Valyi-Nagy *et al.*, 1991). These results suggested that αTIF is dispensable for the establishment of and reactivation from latency.

Although a functional αTIF is dispensable for the estabishment of latency, the cells that harbour latent HSV may not contain the cellular octamer binding protein Oct-1, which is essential for the αTIF to be recruited onto the TAATGARAT element. He *et al.* (1989) reported that Oct-1 expression was not detectable in adult mouse sensory neurons. However, it has been shown that peripheral virus infection may induce Oct-1 expression in some neurons which innervate the site of inoculation (Valyi-Nagy *et al.,* 1991). This induction may supply a population of neurons which are more susceptible to viral replication during the productive phase of infection. It has also been suggested that the TAATGARAT motif may play a role in the establishment of latency. In this respect, Kemp *et al.* (1990) showed that in mouse neuroblastoma cells, a cellular octamer binding protein (Oct-2) could prevent the initiation of HSV productive infection by repressing the activity of α gene promoters. This repression was specific to cells of neuronal origin. Therefore, if present in neurons *in vivo*, Oct-2 mediated repression of HSV replication may facilitate the establishment of latency (Kemp *et al.,* 1990; Lillycrop *et al.,* 1991, 1993, 1994).

During latency, there is limited transcription from one region of the HSV genome which encodes latency associated transcripts (LATs; see section 1.4.3). LAT deficient HSV mutants have been shown to replicate efficiently during productive infection of mice, and virus can be reactivated from sensory ganglia at latent timepoints by explant culture (Javier *et al.,* 1988; Dobson *et al.,* 1989;

Ho & Mocarski, 1989; Steiner *et al.,* 1989; Block *et al.,* 1990). The amount of viral DNA recovered from these ganglia is also indistinguishable from that of latent infection established by wild-type virus (Sedarati *et al.,* 1989; Mitchell *et al.,* 1990 b). In addition, a LAT deficient mutant containing the *Escherichia coli LacZ* gene under the control of the LAT promoter has been shown to express β-galactosidase in neurons of mice following recovery from productive infection (Ho & Mocarski, 1989). These studies suggest that expression of LATs is not required for the establishment of latent infection, although they may function to promote site dependent latent infection because LAT deficient mutants establish latency with greater efficiency in lumbosacral ganglia than in trigeminal ganglia (Sawtell & Thompson, 1992).

Requirements for the establishment of latency have also been studied using a wild-type, neurovirulent HSV-1 strain (SC16). Utilizing a mouse flank inoculation model, Speck and Simmons (1991) were able to demonstrate latent infection (determined by the presence of LAT +ve neurons and the ability to reactivate virus by explant culture) in mouse spinal ganglia in which productive HSV infection had never been detected. In a subsequent study, it was shown that productively infected (viral antigen +ve) as well as latently infected (LAT +ve, viral antigen -ve) neurons appeared synchronously in spinal ganglia during early stages of acute infection with HSV-1 strain SC16 (Speck & Simmons, 1992). It was concluded from these studies that latent infection of a replication-competent HSV could be established without a preceding

productive infection.

In summary, there is overwhelming evidence suggesting that the pathways leading to productive and latent infection can diverge at a very early stage and that latency can occur before significant expression of any virally encoded genes.

1.4.2 Characteristics of latent viral DNA

During latent infection, the viral genome is maintained in a non-replicating state in sensory ganglia (Puga *et al.*, 1978). Latent HSV DNA is structurally different to DNA extracted during productive infection. By Southern blot hybridization, it was shown that during latent infection viral DNA recovered from brains and ganglia of mice and humans lacked detectable genomic termini (Rock & Fraser, 1983; Efstathiou *et al.*, 1986). The lack of genomic termini was shown to be due to the joining of the termini to form a fragment equivalent to the virion junction fragment (Rock & Fraser, 1985). The presence of an "endless" form of viral DNA is consistent with a circular, concatemeric or integrated configuration, and not unit-length DNA.

Using density gradient centrifugation, Mellerick and Fraser (1987) showed that the majority of latent viral DNA could be separated from host chromosomal DNA and therefore existed in an episomal state. However, this report could not exclude the possibility that a small proportion of latent viral genomes were

integrated into cellular DNA sequences.

HSV DNA from the brains of latently infected mice has been shown to be tightly packaged with histones in a nucleosomal structure similiar to that of cellular chromatin and it has been suggested that such an association may play a role in the regulation of viral gene expression during latency (Deshmane & Fraser, 1989). Further, it has been proposed that methylation of latent HSV DNA may also play a role in maintaining the virus genome in an inactive state (Youssoufian *et al.,* 1982; Stephanopoulos *et al.,* 1988; Bernstein & Kappes, 1988; Whitby *et al.,* 1988).

1.4.3 Latency associated transcription

During latency the majority of the genome appears to be transcriptionally silent. However, transcripts derived from a limited region of the genome are detectable in latently infected ganglia by *in situ* and northern blot hybridization (Croen *et al.,* 1987; Deatly *et al.*, 1987; Rock *et al.,* 1987 b; Stevens *et al.*, 1987; Gordon *et al.,* 1988; Krause *et al.,* 1988; Steiner *et al.,* 1988; Wechsler *et al.,* 1988; Wagner *et al.,* 1988 b; Cook *et al.,* 1991). During latent infection, latency associated transcripts (LATs) are confined to nuclei of sensory neurons (Rock *et al.*, 1987 b; Spivack & Fraser, 1987; Stevens *et al.*, 1987).

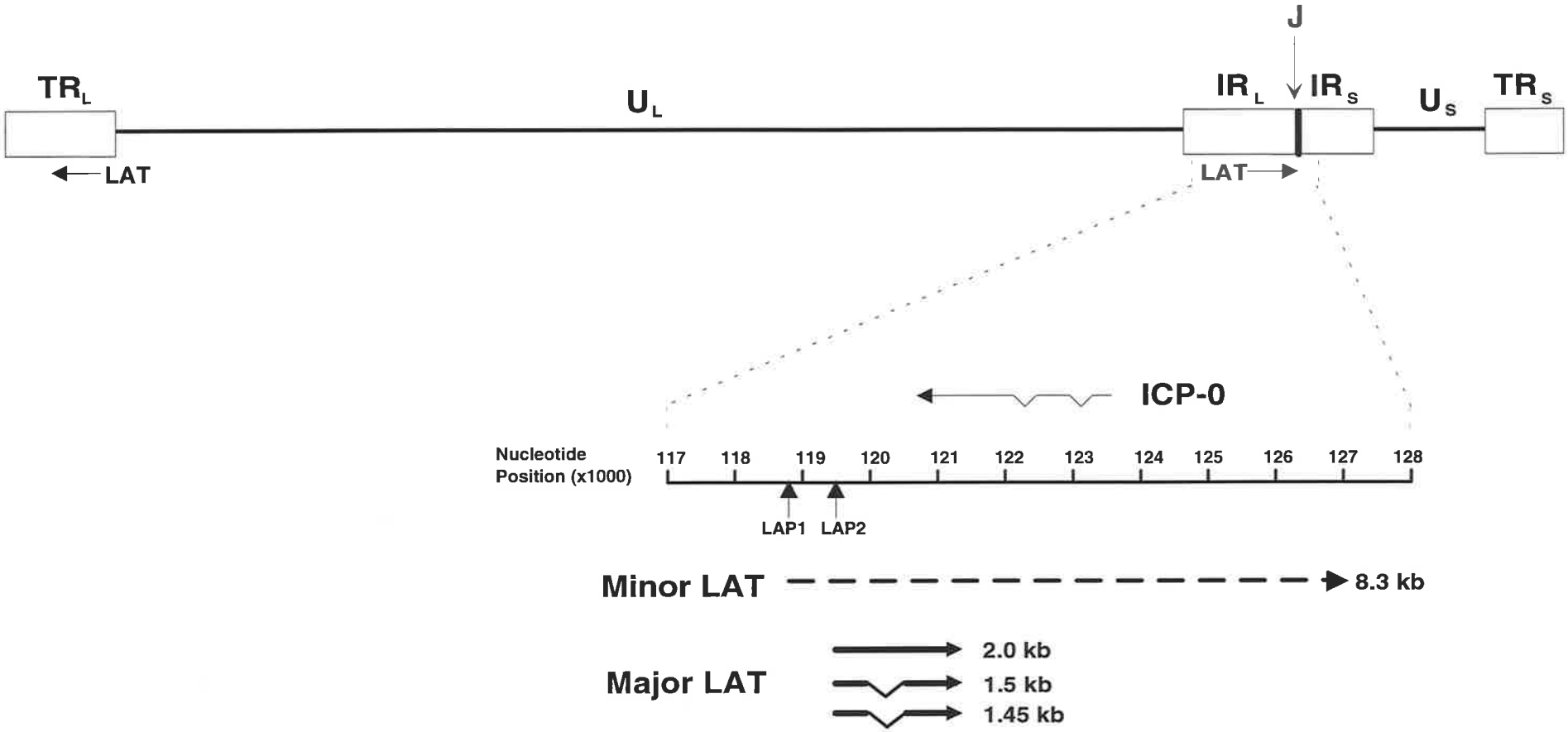
HSV-1 LATs map to the inverted repeat sequences flanking U_L and partially overlap and are complementary to the ICP0 transcript (Croen *et al.,* 1987; Rock *et al.,* 1987 b; Stevens *et al.,* 1987; Spivack & Fraser, 1987; Krause *et al.,* 1988) (Figure 1.3). LATs have been identified during latent infections with HSV-2 (Suzak & Martin, 1989; Mitchell *et al.,* 1990 a; Krause *et al.,* 1991; Tenser *et al.,* 1991), bovine herpes virus type 1 (Rock *et al.,* 1987 a; Homan & Easterday, 1980, 1983; Kutish *et al.,* 1990) and PRV (Cheung *et al.,* 1989; Lokensgard *et al.,* 1990; Priola *et al.,* 1990).

1.4.3.1 Characteristics of LATs

Up to 3 collinear LATs, with approximate sizes of 2.0kb, 1.5kb and 1.45kb, have been detected by northern blot analysis of RNA extracted from latently infected ganglia (Rock *et al.,* 1987 b; Spivack & Fraser, 1987, 1988 a, b; Krause *et al.,* 1988, Wagner *et al.,* 1988 b; Wechsler *et al.,* 1988). These abundant transcripts are commonly referred to as major LATs (Figure 1.3). Although HSV-1 major LATs contain two open reading frames (ORFs) they are not polyadenylated and are confined to nuclei of latently infected neurons (Stevens *et al.,* 1987; Rock *et al.,* 1987 b; Spivack & Fraser, 1987; Wagner *et al.,* 1988 a). The predominant species, constituting 50-90% of the major LATs (depending on the virus strain) is a 2.0kb transcript (Wagner *et al.,* 1988 a, b). The 1.5kb and 1.45kb major LAT species are thought to be derived from the 2.0kb RNA by splicing (Deatly *et al.,* 1988; Wagner *et al.,* 1988 b; Wechsler *et al.,* 1988; Mitchell *et al.,* 1990 b; Spivack *et al.,* 1991).

Figure 1.3

Genome structure of HSV-1 showing the region from which LATs are transcribed. Major LATs (approximately 1.45-2.0kb) are shown as thick arrows. Minor LAT (approximately 8.3kb) is shown as a broken arrowed line. The expanded view of the internal repeat regions (from 117-128kb) shows the relative positions of the LAT promoter regions (LAP1 and LAP2). The transcript encoding ICP0 (thin arrow) overlaps and is transcribed in the opposite direction to LATs. Also depicted are the long unique region (U_L), short unique region (U_S), long terminal repeat region (TR_L), long internal repeat region (IR_L), short terminal repeat region (TR_S), short internal repeat region (IR_S) and the junction between the L and S segments (J).


J
TR L
U L
IR L
IR S
U S
TR S
LAT
LAT
ICP-0
Nucleotide Position (x1000)
117
118
119
120
121
122
123
124
125
126
127
128
LAP1
LAP2
Minor LAT
8.3 kb
Major LAT
2.0 kb
1.5 kb
1.45 kb

It has been suggested that major LATs may be stable introns derived from a larger, less abundant transcript (Dobson *et al.,* 1989; Zwaagstra *et al.,* 1990; Farrell *et al.,* 1991). This is supported by several observations:

(i) *In situ* hybridization studies of latently infected tissues have detected transcripts of low abundance, designated minor LATs, arising from the region between the LAT promoter (LAP1) and the 5' terminus of major LATs and also extending downstream of major LATs (Deatly *et al.,* 1987; Rock *et al.,* 1987 b; Krause *et al.,* 1988; Wagner *et al.,* 1988 a; Wechsler *et al.,* 1988; Dobson *et al.,* 1989; Mitchell *et al.,* 1990 b; Zwaagstra *et al.,* 1990).

(ii) An 8-9kb polyadenylated LAT has been detected by northern blot analysis of RNA isolated from productively infected cells (Dobson *et al.,* 1989; Devi-Rao *et al.,* 1991). In addition, this minor LAT species has also been detected by northern blot analysis of RNA isolated from latently infected rabbit ganglia (Zwaagstra *et al.,* 1990).

(iii) DNA sequence analysis surrounding the major LATs has revealed the presence of consensus splice acceptor and donor sites (Wagner *et al.,* 1988 b) which are active during latency (Farrell *et al.,* 1991).

Thus, minor LATs may represent an unstable primary transcript, which is subsequently spliced and processed to produce the more stable 2.0kb, 1.5kb and 1.45kb major LATs (see Figure 1.3). However, to date a 6-7kb spliced product of this processing event has not been detected.

Sequencing data has indicated that the closest RNA polymerase II LAT promoter consensus sequence, now termed LAP1, is situated approximately 0.7kb upstream of the shared 5' end of the major LATs (Dobson *et al.,* 1989; Zwaagstra *et al.,* 1989, 1990; Batchelor & O'Hare, 1990) (Figure 1.3). This region contains a TATA box, a potential CAAT box and Sp1-binding sites, strongly suggesting that it is a transcriptional promoter (Wechsler *et al.,* 1988, 1989). In addition, this region contains a 55bp ICP4 binding site. Binding of ICP4 causes repression of the LAP1 promoter (Batchelor & O'Hare, 1990; Farrell *et al.*, 1994). Other potential regulatory elements in this region include a cyclic adenosine monophosphate (cAMP) responsive element (Leib *et al.,* 1991), a palindromic sequence which binds to a host cell factor (i.e. LAT promoter binding factor, LPBF) (Zwaagstra *et al.,* 1991) and sequences upstream of the 5' end of major LATs which enhance promoter activity in cells of neuronal origin (Batchelor & O'Hare, 1990, 1992). Overall, these regulatory sequences suggest that LAP1 promoter activity may be affected by several host or viral factors.

The importance of the LAP1 promoter in directing LAT production has been supported by several lines of evidence:

(i) Transient chloroamphenicol acetyl-transferase (CAT) assays have shown that this region confers promoter activity in cells of both neuronal and non-neuronal origin (Batchelor & O'Hare, 1990; Zwaagstra *et al.,* 1990, 1991).

(ii) Several HSV-1 mutants which have deletions encompassing the putative promoter-regulatory domain do not transcribe detectable amounts of major LATs during latent infection (Javier *et al.,* 1988; Dobson *et al.,* 1989; Leib *et al.,* 1989 a; Steiner *et al.,* 1989; Block *et al.,* 1993).

(iii) Insertion of the rabbit β-globin gene into HSV immediately downstream of this promoter results in detectable β-globin transcripts in latently infected ganglia (Dobson *et al.,* 1989).

These observations support the proposal that LAP1 is responsible for LAT synthesis during latency. However, it has now been indicated that LAT transcriptional control may be more complex than described above.

A second putative LAT promoter, designated LAP2, has been identified and partially characterized (Goins *et al.,* 1994). Transient CAT assays were utilized to detect a latency active promoter, 0.19-0.59kb upstream of the 5' end of the

major LATs (Figure 1.3), which promoted RNA synthesis and transcription initiating at or near the 5' end of the major LATs. In contrast to LAP1, this promoter region lacks a TATA box but contains *cis*-acting regulatory elements, including a region with sequence homology to transcription-initiator elements present in some TATA-less promoters. A recent study by Chen *et al.* (1995) demonstrated that both LAP1 and LAP2 are critical for 2.0kb major LAT expression. However, they showed that LAP1 was essential for LAT expression during latency, whereas LAP2 was primarily responsible for LAT expression in lytic infection in cell culture. Thus, LAP1 and LAP2 may be functionally independent promoter elements that control 2.0kb LAT expression during different stages of HSV-1 infection.

1.4.3.2 Function of LATs

The function of LATs has not been defined. Viruses with various mutations and deletions in the LAT region have been characterized. LAT deficient mutants with an intact LAP1 promoter have been shown to reactivate with normal kinetics and efficiency compared with parental or revertant virus strains (Ho & Mocarski, 1989; Izumi *et al.,* 1989; Sedarati *et al.,* 1989; Dobson *et al.,* 1989; Block *et al.,* 1990; Fareed & Spivack, 1994). However, viruses in which the LAP1 promoter has been mutated generally show reduced levels or slower reactivation kinetics *in vitro* (by explant culture) (Leib *et al.,* 1989 a; Steiner *et al.,* 1989; Hill *et al.,* 1990; Mitchell *et al.,* 1990 b; Trousdale *et al.,* 1991; Block *et al.,* 1993; Rader *et al.,* 1993), indicating a possible role for LATs

during reactivation. Interestingly, deletion of the LAP2 promoter, which promotes synthesis of major LATs in productively infected cells, does not inhibit reactivation of virus from mouse neurons (Maggioncalda *et al.*, 1994). Of worthwhile note are the studies using rabbits infected with LAT deficient mutants which have suggested that there may be physiological differences between induced and spontaneous reactivation *in vivo* and that LATs may facilitate the former process (Hill *et al.,* 1990; Trousdale *et al.,* 1991; Perng *et al.,* 1994). It has also been suggested that intracellular levels of cAMP may stimulate reactivation via a function encoded by LATs. This is because (i) a functional cAMP response element (CRE) in the LAT promoter region of HSV-1 has been identified (Leib *et al.,* 1991), (ii) agents that can increase intracellular levels of cAMP can accelerate the reactivation of latent virus in explanted ganglia (Sainz de la Maza *et al.,* 1989; Foster *et al.,* 1989; Leib *et al.*, 1991) and (iii), cAMP analogs or adenylate cyclase activators accelerate reactivation of wild-type HSV but not a LAT deletion mutant (Leib *et al.,* 1991).

Given that the major LATs overlap and are complementary to the 3' end of ICP0 mRNA, it was postulated that LATs may regulate expression of ICP0 by an anti-sense RNA interference mechanism (Stevens *et al.,* 1987). As discussed earlier, ICP0 is very important in early stages of productive infection for the transactivation of β and γ genes. Hence, suppression of $\alpha 0$ by binding of LAT RNA might regulate two aspects of HSV infection. Firstly, $\alpha 0$ repression could

potentially shut off viral replication, promoting the establishment of latency. This hypothesis is not supported by the finding that LAT deficient HSV mutants retain the ability to establish and maintain latent infections (Ho & Mocarski, 1989; Leib *et al.,* 1989 a; Sedarati *et al.,* 1989; Steiner *et al.,* 1989; Block *et al.,* 1990; Mitchell *et al.,* 1990 b). Secondly, antisense repression of $\alpha 0$ by LATs could play a crucial role in regulating virus reactivation, because $\alpha 0$ deficient HSV mutants establish latency but reactivate poorly (Leib *et al.,* 1989 b). If this were the case, LAT deficient mutants would be expected to reactivate more readily than wild-type virus. However, as discussed above, LAT deficient viruses reactivate with similiar or lower efficiency than wild-type viruses, suggesting that accumulation of LATs may promote rather than repress reactivation by providing a large pool of viral transcripts that are immediately available when the conditions for HSV replication arise (Steiner *et al.,* 1989).

The HSV-1 major LAT sequence has two potential open reading frames (ORF1 and ORF2), of which ORF2 is conserved between several HSV-1 strains (Wagner *et al.,* 1988 a; Wechsler *et al.,* 1989; Spivack *et al.,* 1991). However, there is little conservation between HSV-1 and HSV-2 (Mitchell *et al.,* 1990 a) or between HSV-1 and PRV (Cheung, 1989, 1990). Moreover, ORF1 and ORF2 do not exhibit the codon usage common for other HSV proteins (McGeoch *et al.,* 1991) and there are frameshift differences in LAT sequences of different strains of HSV-1 (Perry & McGeoch, 1988; McGeoch *et*

al., 1991). These observations, together with the lack of extensive polyadenylation of major LATs (Rock *et al.,* 1987 b; Spivack & Fraser, 1987; Wagner *et al.,* 1988 b) and their nuclear localization, suggest that major LATs do not encode a protein. In addition, antisera raised against synthetic polypeptides potentially encoded by the major LAT region have failed to detect a protein product in latently infected ganglia (Wagner *et al.,* 1988 a; Wechsler *et al.,* 1989).

However, Doerig *et al.* (1991) bacterially expressed a fusion protein containing part of ORF2 within the 2.0kb LAT species and raised antisera to this chimeric product. The resulting antisera detected latency associated antigens (LAA) of approximately 80kDa and 45kDa in neurons latently infected *in vitro.* However, the significance of these LAAs is difficult to understand because LAAs are much larger than the ~33kDa polpeptide predicted from the ORF2 sequence (Wechsler *et al.,* 1989; McGeoch *et al.,* 1991; Spivack *et al.,* 1991) and LAAs have not been detected during latent infection *in vivo.*

The fact that the minor LAT species is polyadenylated suggests that it may encode a protein (Dobson *et al.*, 1989; Zwaagstra *et al.*, 1990). In this respect, Lagunoff and Roizman (1994) identified within the minor LAT region an open reading frame (ORF P), antisense to the $\gamma_1$34.5 gene, which expressed a protein. ORF P expression occurred in the absence of prior viral protein synthesis and was strongly repressed by ICP4 (Yeh & Schaffer, 1993;

Lagunoff & Roizman, 1995). Further, the ORF P protein has been shown to accumulate in nuclei of infected cells *in vitro* (Lagunoff & Roizman, 1995). The ORF P transcript is made in very small amounts and attempts to detect it in latently infected neurons have, to date, been unsuccessful (Yeh & Schaffer, 1993). Although the specific role of ORF P protein during latency has not been tested, the properties of ORF P elucidated to date are consistent with the hypothesis that a virally encoded protein may play a role during latent infection.

The non-essential nature of LATs for virus replication and establishment of latency makes the LAT locus a convenient site for the introduction of foreign genes into the HSV genome. In the work described in this thesis a recombinant virus containing a MHC class I gene inserted into the major LAT region was used to study MHC class I expression in HSV infected neurons.

1.4.4 Other viral transcripts found in latently infected ganglia

In addition to LATs, it has recently been reported that certain mRNAs specific for productive-cycle genes are present in mouse ganglia latently infected with HSV-1. Using a quantitative PCR assay, Kramer and Coen (1995) detected ICP4-specific mRNA and TK mRNA (whose transcription in productive infection is dependent on ICP4) in latently infected ganglia. Compared with LATs (average $4x10^4$ molecules per viral genome), the amounts of these transcripts was small; 1-7 molecules and 500 molecules of ICP4 and TK-

specific RNA per viral genome, respectively. These results suggest that latency is a more dynamic process than originally envisaged and Kramer & Coen (1995) suggest that this low level gene expression may play an integral role in the molecular events leading to successful viral reactivation.

1.5 Immune responses to HSV infection

Non-adaptive and adaptive immune responses are activated during the course of HSV infection. The relative roles of these immune effector mechanisms are discussed below. Despite detailed studies on antibody and T cell responses in humans, the majority of available information on the relative importance of particular immunological mechanisms in immunity to HSV has been extrapolated from experimental animal models.

1.5.1 Non-adaptive immunity

The early host response to HSV is non-adaptive or innate. Macrophages, α/β interferon (α/β-IFN), NK cells and other factors become active during the first few hours and days after infection and function to restrict virus replication and spread (Lopez, 1985).

The importance of macrophages as a first line of defence against HSV has been shown in several studies. For instance, neonatal mice, which are very susceptible to HSV infection, can be protected by the adoptive transfer of

macrophages from HSV infected adult mice (Johnson, 1964). Further, depletion of tissue macrophages from adult mice increases susceptibility to HSV infection (Morahan & Morse, 1979; Pinto *et al.,* 1991). This effect was shown to be enhanced when (i) tissue macrophages rather than circulating monocytes were depleted, (ii) tissue macrophages around the site of infection were destroyed prior to or after infection and (iii), macrophages were destroyed rather than functionally inhibited (Morahan *et al.,* 1986; Pinto *et al.,* 1991). HSV can spread to neighbouring cells by an extracellular route or to adjacent cells by intercellular bridges. Lodmell *et al.* (1973) reported inhibition of direct HSV spread between cells by activated peritoneal macrophages. These cells have been shown to inhibit viral replication (Morahan *et al.,* 1980) and virus spread (Hayashi *et al.,* 1980) at an early stage of infection and their antiviral activity was independent of antibodies or the immune status of the mice.

α/β-IFN production is an early indicator of virus infection, with levels in the serum peaking 2 days after infection. These molecules have multiple effects, including inhibition of virus replication and activation of NK cells and macrophages (Joklik, 1990). The significance of α/β-IFN in resistance to HSV infection was demonstrated in mice treated with antibodies which neutralize IFN activity, which rendered the animals more susceptible to HSV infection than untreated controls (Gresser *et al.,* 1976).

There are numerous reports that γ interferon (γ-IFN), secreted by activated T cells, controls acute viral infections (Klavinskis *et al.,* 1989; Leist *et al.,* 1989 b; Gessner *et al.,* 1990; Karupiah *et al.,* 1990, 1993; Raniero *et al.,* 1990; Hendricks *et al.,* 1992; Lucin *et al.,* 1992; Ramshaw *et al.,* 1992; Guidotti *et al.,* 1994). Smith *et al.* (1994) showed that during the course of acute HSV infection, mice treated with anti-γ-IFN monoclonal antibodies had a decreased ability to eliminate HSV from infected skin. In addition, neutralizing anti-γ-IFN monoclonal antibody treatment substantially diminished the ability of immune cells from infected donors to adoptively transfer immunity to recipient mice challenged with HSV (Smith *et al.,* 1994).

Yamamoto *et al.* (1993) demonstrated a potential role for γ-IFN during the early stages of primary HSV infection in humans. γ-IFN and NK cell levels were shown to increase shortly after primary infection and decrease with the disappearance of oral inflammation. In addition, Burchett *et al.* (1992) suggested that decreased γ-IFN production during early stages of HSV infection in neonates and pregnant women may directly influence the severity of clinical disease.

Recently, Cantin *et al.* (1995) demonstrated that during acute HSV infection, viral replication was enhanced in trigeminal ganglia and brainstems of mice from which γ-IFN was ablated by monoclonal antibody treatment and in mice genetically lacking the γ-IFN receptor (Rgko mice). Secreted γ-IFN was

detected up to 6 months after HSV infection in areas of $CD4^+$ and $CD8^+$ T cell infiltration. The detection of prolonged γ-IFN secretion during latency raises the possibility that γ-IFN and perhaps other cytokines modulate reactivated HSV infection. In support of this proposal, clinical studies have shown that the interval between recurrrent infections is greater in individuals with higher levels of (i) γ-IFN in recurrent lesions (Torseth & Merigan, 1986) or (ii) γ-IFN secreted by *in vitro* stimulated peripheral blood mononuclear cells (Cunningham & Merigan, 1983).

In addition to γ-IFN, other cytokines may contribute to the clearance of HSV. For instance, Chan *et al.* (1990) demonstrated that IL-3 plays a role in protection of mice against lethal HSV infection. Further, TNF-α has been shown to inhibit HSV replication *in vitro* and *in vivo* (Wong & Goeddel, 1986; Rossol-Voth *et al.,* 1991). The activity of TNF-α was synergisitic with that of γ-IFN (Nguyen *et al.,* 1994).

The precise role of NK cells in the host response to HSV infection remains unclear. Early studies by Lopez *et al.* (1980) indicated that resistance to HSV infection was reduced by depletion of NK cells *in vivo*, implicating NK cells as a major factor in resistance. In addition, a young patient with a defect in NK cell activity had an increased susceptibility to HSV infection (Biron *et al.,* 1989). This observation in a person with a very rare genetic defect supported the notion that human NK cells can potentially act as a first line defence

immune mechanism against HSV. However, NK deficient beige mice and mice depleted of NK cells *in vivo* with anti-NK 1.1 or anti-asialo-GM1 antibodies are no more susceptible to HSV than mice with normal NK cell activity (Bukowski & Welsh, 1986). Finally, adoptive cell transfer studies have indicated that NK cells are not required in the natural resistance of normal mice to HSV infections (Bukowski & Welsh, 1986).

1.5.2 Adaptive (antigen-specific) immunity

Antigen-specific immunity to HSV comprises of humoral and T cell mediated immune mechanisms. Each component plays a different role in the control of and recovery from HSV infection.

1.5.2.1 Antibody mediated immunity

The role of antibodies in protection against and control of HSV infection has been studied extensively. There are several lines of evidence which suggest that the antibody response contributes little to controlling cutaneous HSV infection.

(i) Passive transfer of anti-HSV antibodies into athymic nude mice before infection fails to eliminate infectious virus from the skin, but limits the spread of virus to sensory ganglia (Kapoor *et al.,* 1982 a). However, interpretation of these experiments is complicated by the fact that during primary HSV infection, virus reaches the nervous system before the development of a natural

antibody response (Morahan *et al.,* 1981). Hence, alternative studies have used B cell suppressed (agammaglobulinaemic) mice.

(ii) Aggamaglobulinaemic mice clear infection from the skin with normal kinetics but spread of virus to or within the nervous system is enhanced, as is the incidence of latent infection (Kapoor *et al.,* 1982 b; Simmons & Nash, 1987). The insignificant role of antibody in controlling cutaneous HSV infection is concordant with the clinical observation that patients with aggamaglobulinaemia control HSV satisfactorily (Merigan & Stevens, 1971).

The effector mechanisms mediated by antibodies against HSV include antibody-dependent cell cytotoxicity (ADCC) and neutralization. In humans, three cell types, namely macrophages, neutrophils and NK cells found in peripheral blood, mediate ADCC *in vitro*. The importance of this mechanism *in vivo* has been shown by various studies in mice. Firstly, HSV infection can be prevented in immunocompromised or neonatal mice by the transfer of antibody and non-immune spleen cells but not by antibody alone (Kohl & Loo, 1982). Secondly, mice can be protected by the passive transfer of non-neutralizing monoclonal antibodies, which mediate ADCC *in vitro* (Rector *et al.,* 1982).

Neutralizing antibodies, which are active against cell-free virions, may be an important mechanism by which antibody mediates its protective effect in the nervous system, possibly by preventing the spread of virus from epithelial cells to nerve endings. *In vitro,* neutralizing antibodies have little effect on the replication and spread of HSV, because HSV can spread from cell to cell by forming syncytia (Hooks *et al.,* 1976). In peripheral tissues there is histological evidence of syncytium formation, suggesting that neutralizing antibodies may not have access to virus in the skin. This may explain why B cell suppression does not result in a more florid cutaneous infection. However, cell fusion is not a feature of HSV infection in the peripheral nervous system (PNS) and it has been proposed that virus spreads between neurons via an extracellular route (Simmons & Nash, 1985).

Passive transfer of neutralizing but not non-neutralizing anti-HSV monoclonal antibodies reduces the severity of neural infection, provided the antibody is administered prior to virus inoculation (Simmons & Nash, 1985). If neutralizing antibodies are able to interrupt virus access to the nervous system, then it is plausible that antibodies could also neutralize virus emerging from nerve endings following reactivation of latent infection, thereby preventing re-infection of epithelial cells. Support for this hypothesis was provided by Simmons & Nash (1985) using a mouse flank zosteriform model (Simmons & Nash, 1984). Neutralizing antibodies prevented zosteriform spread of virus but the amount of antibody required was several times that found in serum of mice

following natural infection.

1.5.2.2 T cell mediated immunity

There are several lines of evidence suggesting that T cell responses are involved in the control of HSV infection.

(i) Primary and recurrent HSV infections are usually more severe in T cell immunodeficient humans compared with those who are immunocompetent (Merigan & Stevens, 1971).

(ii) Neonatally thymectomized and congenitally athymic mice die following cutaneous inoculation of HSV (Mori *et al.,* 1967; Nagafuchi *et al.,* 1979; Kapoor *et al.,* 1982 a).

(iii) Spleen or lymph node cells from HSV infected mice, when transferred to naive nude mice or mice immunosuppressed by irradiation (Oakes, 1975) or cyclophosphamide (Rager-Zisman & Allison, 1976), confer protection against lethal challenge with HSV. Protection is abolished by pretreatment of the donor cells with anti-Thy.1 antibodies and complement, indicating that T cells are an essential component of the transferred population (Rager-Zisman & Allison, 1976).

(iv) *In vivo* depletion of T cells using anti-lymphocyte serum (Oakes, 1975) or monoclonal antibodies (Simmons, 1985; Smith *et al.,* 1994) greatly reduces the survival of HSV infected mice, indicating that T cells are required for the resolution of infection.

The protective T cell population is comprised of major histocompatibility complex (MHC) class I restricted ($CD8^+$) and MHC class II restricted ($CD4^+$) T lymphocytes. This is consistent with experiments showing that $CD4^+$ (Nagafuchi *et al.,* 1982; Nash & Gell, 1983; Leung *et al.,* 1984) and $CD8^+$ (Larsen *et al.,* 1983; Sethi *et al.,* 1983; Bonneau & Jennings, 1989, 1990) enriched populations are capable of transferring anti-HSV immunity to naive mice.

There is extensive evidence suggesting that $CD4^+$ T cells play a central role in host recovery from an HSV infection. The lymph nodes of HSV infected mice, as early as 4 days after infection, contain activated $CD4^+$ T cells which have the ability to adoptively transfer delayed type hypersensitivity (DTH) to infected recipients (Nash & Gell, 1983). Such T cells promote rapid clearance of infectious virus from epidermal surfaces. Their effect may be mediated by recruitment and activation of macrophages (Nash & Cambouropoulus, 1993). Nash *et al.* (1980 a) reported that a DTH response to HSV was associated with reduced virus replication in the skin, which affected the amount of virus spreading to the PNS. In addition, depletion of $CD4^+$ T cells was shown to

delay clearance of virus from cutaneous lesions, supporting a role for $CD4^+$ T cells in the skin. However, it has been shown that anti-HSV immunity can be transferred without inducing DTH (Nash *et al.,* 1981; Larsen *et al.,* 1983) and that rapid clearance of virus from the inoculation site following adoptive transfer of primed T cells requires compatibility at MHC class I and class II loci (Nash *et al.,* 1981). Therefore, a DTH response is not the sole mechanism by which HSV is controlled.

Mice infected with HSV generate $CD8^+$ cytotoxic T cells which are found both in draining lymph nodes (Pfizenmaier *et al.,* 1977 a, b) and spleen (Lawman *et al.,* 1980). CTLs have been shown to contribute to the control of HSV infection and disease when adoptively transferred into infected recipients (Larsen *et al.,* 1983; Sethi *et al.,* 1983; Bonneau & Jennings, 1989, 1990). To demonstrate cytotoxicity *in vitro*, lymph node or spleen cells from HSV infected mice must be cultured for several days prior to incubation with target cells (Pfizenmaier *et al.,* 1977 a; Nash *et al.,* 1980 b). The reason for this is unknown, but it has been suggested that *in vivo*, development of HSV specific CTLs is inhibited by the presence of suppressor cells (Horohov *et al.*, 1986).

The function of $CD8^+$ T cells in the control of HSV infection appears to be less important in the skin than in the PNS. For instance, the rate of HSV clearance from the skin is unaltered by $CD8^+$ T cell depletion (Nash *et al.*, 1987; Simmons & Tscharke, 1992; Smith *et al.*, 1994). However, it is important to note that the contribution of $CD8^+$ T cells to the control of cutaneous HSV infection is strongly influenced by the MHC genotype of the host (Simmons & Tscharke, 1992).

There is strong evidence to suggest that $CD8^+$ T cells play a crucial role in the clearance of HSV from the PNS. In mice depleted of $CD8^+$ T cells, a high proportion of HSV infected neurons are killed and the spread of virus in the PNS is enhanced (Simmons & Tscharke, 1992). The mechanism by which $CD8^+$ T cells contribute to the control of HSV infection in the nervous system is not clear. However, it has been proposed that $CD8^+$ T cells are able to exert an anti-HSV effect in the PNS not by lysis of the infected cell, but rather via anti-viral cytokine release. This is supported by many examples of $CD8^+$ T cell clones that can secrete lymphokines in an antigen-specific manner *in vitro* (Yasukawa & Zarling, 1984; Johnson *et al.*, 1990).

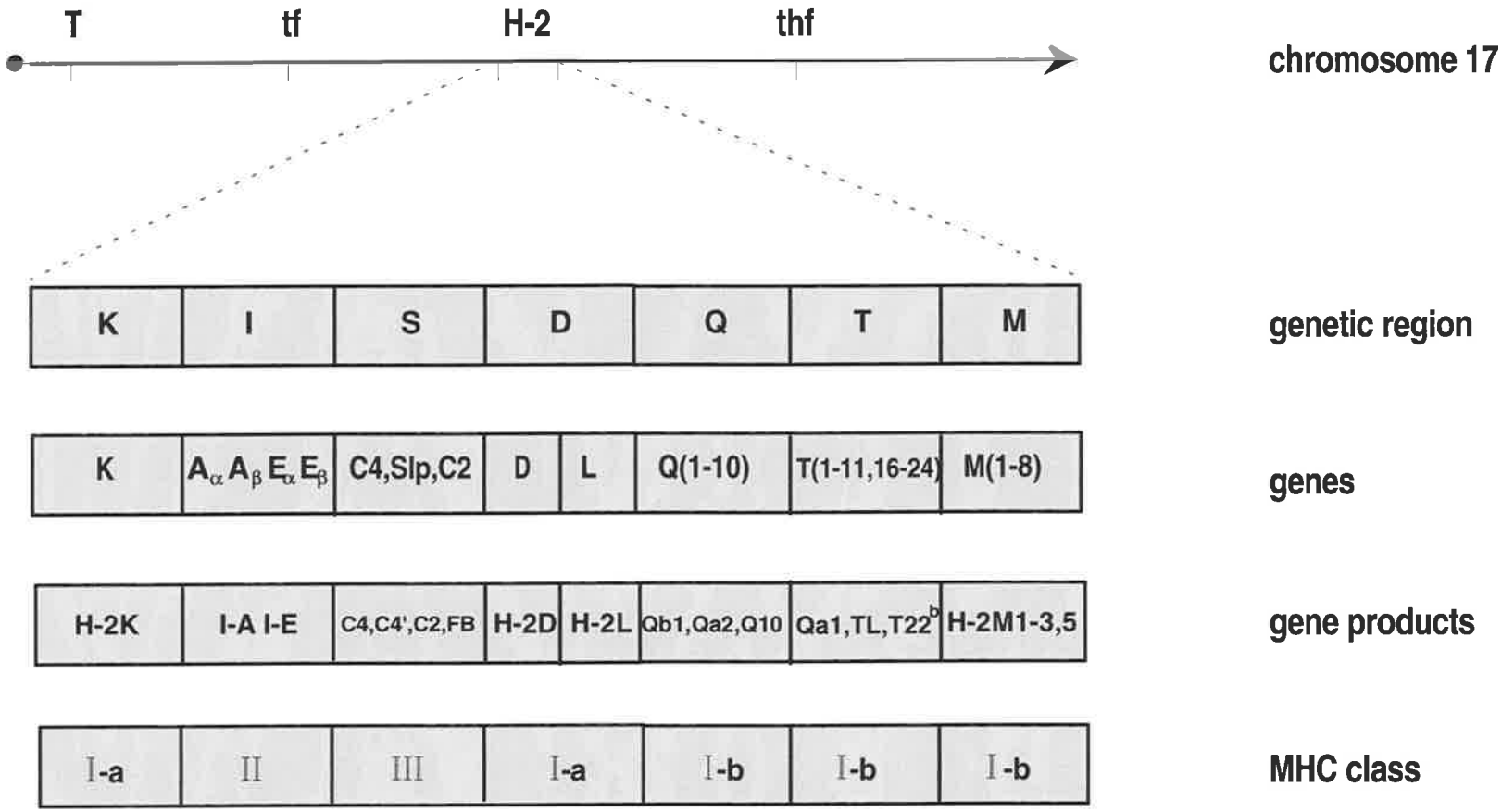
1.6 The major histocompatibility complex (MHC)

The MHC of mammals is a multigene family whose members encode cell-surface glycoproteins involved in immune recognition and response to foreign antigens. The MHC has been highly conserved throughout vertebrate evolution and the MHCs of mice (H-2) and humans (HLA) have been extensively studied. In humans, the HLA complex resides on chromosome 6. The H-2 complex of the mouse resides on chromosome 17. On the basis of structure and function the MHC has been divided into two regions, called class I and class II (Klein, 1975; Snell *et al.,* 1975; Klein *et al.,* 1981; Hood *et al.,* 1983; Flavell *et al.,* 1985).

In mice, class I MHC genes are located in five discrete genetic loci: H-2K, H-2D/H-2L, Q, T and M. Class II MHC genes are located in two genetic loci, H-2I-A and H-2I-E, that map between H-2K and H-2D/H-2L (Figure 1.4). Mouse MHC H-2K, H-2D and H-2L genes and their products (referred to here as MHC-Ia molecules) have been extensively characterized. H-2K, H-2D, and H-2L molecules are highly polymorphic (Klein *et al.,* 1983; Klein, 1986; Lawlor *et al.,* 1990). Their sole function appears to be transport of antigenic peptides, derived from degraded cytoplasmic proteins, to the cell-surface, allowing $CD8^+$ T cells to identify cells containing foreign antigens (Zinkernagel & Doherty, 1974; Marrack & Kappler, 1987). MHC-Ia antigens are expressed on virtually all vertebrate nucleated cell types, although unusually low levels are expressed

Figure 1.4

Map of the murine MHC complex encoded on chromosome 17. Classical class I-a molecules are encoded by 2 genetic regions (K & D). Non-classical class I-b molecules are encoded by 3 genetic regions (Q, T & M). The genetic regions I and S encode MHC class II antigens and components of the complement system, respectively. The genes and gene products of MHC class I-a, I-b, II and III are shown. Figure adapted from Roitt (1988) and Shawar *et al.* (1994) and is not to scale.


T
tf
H-2
thf
chromosome 17
K
I
S
D
Q
T
M
genetic region
K
A_α A_β E_α E_β
C4,Slp,C2
D
L
Q(1-10)
T(1-11,16-24)
M(1-8)
genes
H-2K
I-A I-E
C4,C4',C2,FB
H-2D
H-2L
Qb1,Qa2,Q10
Qa1,TL,T22^b
H-2M1-3,5
gene products
I-a
II
III
I-a
I-b
I-b
I-b
MHC class

by neurons (Berah *et al.*, 1970; Edidin, 1972; Schachner & Siedman, 1973; Schachner & Hammerling, 1974; Vitetta & Capra, 1978; Williams *et al.*, 1980; Daar *et al.*, 1984; Wong *et al.*, 1984, 1985; Bartlett *et al.*, 1989; Joly *et al.*, 1991) (see section 1.7).

Products of the Q, T and M regions (referred to here as MHC-Ib molecules) are less polymorphic than MHC-Ia gene products (Tewarson *et al.*, 1983; Winoto *et al.*, 1983; Tine *et al.*, 1990) and expression of MHC-Ib molecules is highly tissue specific (Flaherty, 1981; Cosman *et al.*, 1982; Mellor *et al.*, 1984). The functions of MHC-Ib molecules are not known (Shawar *et al.*, 1994).

Class II MHC antigens encoded by H-2I region genes (McDevitt *et al.*, 1972) are highly polymorphic (Shreffler & David, 1975) and are expressed predominantly on the surface of B lymphocytes (Sachs & Cone, 1973; Hammerling *et al.*, 1974; Frehlinger *et al.*, 1974), macrophages (Cowing *et al.*, 1979; Beller & Unanue, 1980), dendritic cells (Steinman, 1981) and certain epithelial cells (Parr & McKenzie, 1979; Klein & Hauptfeld, 1976; McDevitt *et al.*, 1976; Schwartz *et al.*, 1976). Class II molecules function as antigen-specific receptors for helper $CD4^+$ T cells that are required for the generation of CTLs and for antibody production by B cells (Erb & Feldmann, 1975; Kappler & Marrack, 1976; Sprent, 1978).

1.6.1 Structure of MHC-Ia molecules

MHC-Ia molecules are discussed here in detail because this project focuses on MHC-Ia expression on neurons of mice. MHC-Ia antigens are transmembrane molecules consisting of two non-covalently bound glycoproteins, called heavy and light chain in reference to their differing molecular weights (Cresswell *et al.,* 1973) (Figure 1.5). Heavy chains (αCs) (44kDa) are polymorphic glycoproteins encoded by MHC-Ia genes (Coligan *et al.,* 1981). αC genes have 8 exons, each encoding a distinct protein domain (Steinmetz *et al.,* 1981; Moore *et al.,* 1982; Sodoyer *et al.,* 1984; Strachan *et al.,* 1984; Kindt & Singer, 1987) (Figure 1.6). The first exon encodes a leader sequence which targets newly synthesized αCs to the endoplasmic reticulum (ER) and is cleaved from the mature protein. Exons 2, 3, and 4 encode extracellular domains called α1, α2 and α3, respectively. Exon 5 encodes a hydrophobic transmembrane region of the protein and exons 6, 7 and 8 encode a cytoplasmic domain (Flavell *et al.,* 1986).

The light chain, β_2-microglobulin (β_2m) (12kDa), is a non-MHC encoded (Goodfellow *et al.,* 1975; Michaelson, 1981), non-polymorphic protein (Michaelson *et al.*, 1980) which is non-covalently bound to the MHC-Ia αC extracellular domain (Coligan *et al.,* 1981; Yokoyama & Nathenson, 1983) (Figure 1.5). The β_2m gene, located on chromosome 2 in mice and chromosome 15 in humans, consists of 3 exons (Figure 1.6). The first exon encodes a 5' untranslated region and leader sequence. Exon 2 encodes the bulk

Figure 1.5

Schematic structure of a cell-surface MHC-Ia molecule which is comprised of a heavy chain (αC), light chain (β_2m) and an antigenic peptide. The αC consists of 3 extracellular domains (α1, α2, α3), a single plasma membrane domain and a cytoplasmic domain. The peptide binding site is between α1 and α2 and β_2m forms extensive contacts with the αC. Figure adapted from Roitt (1988).

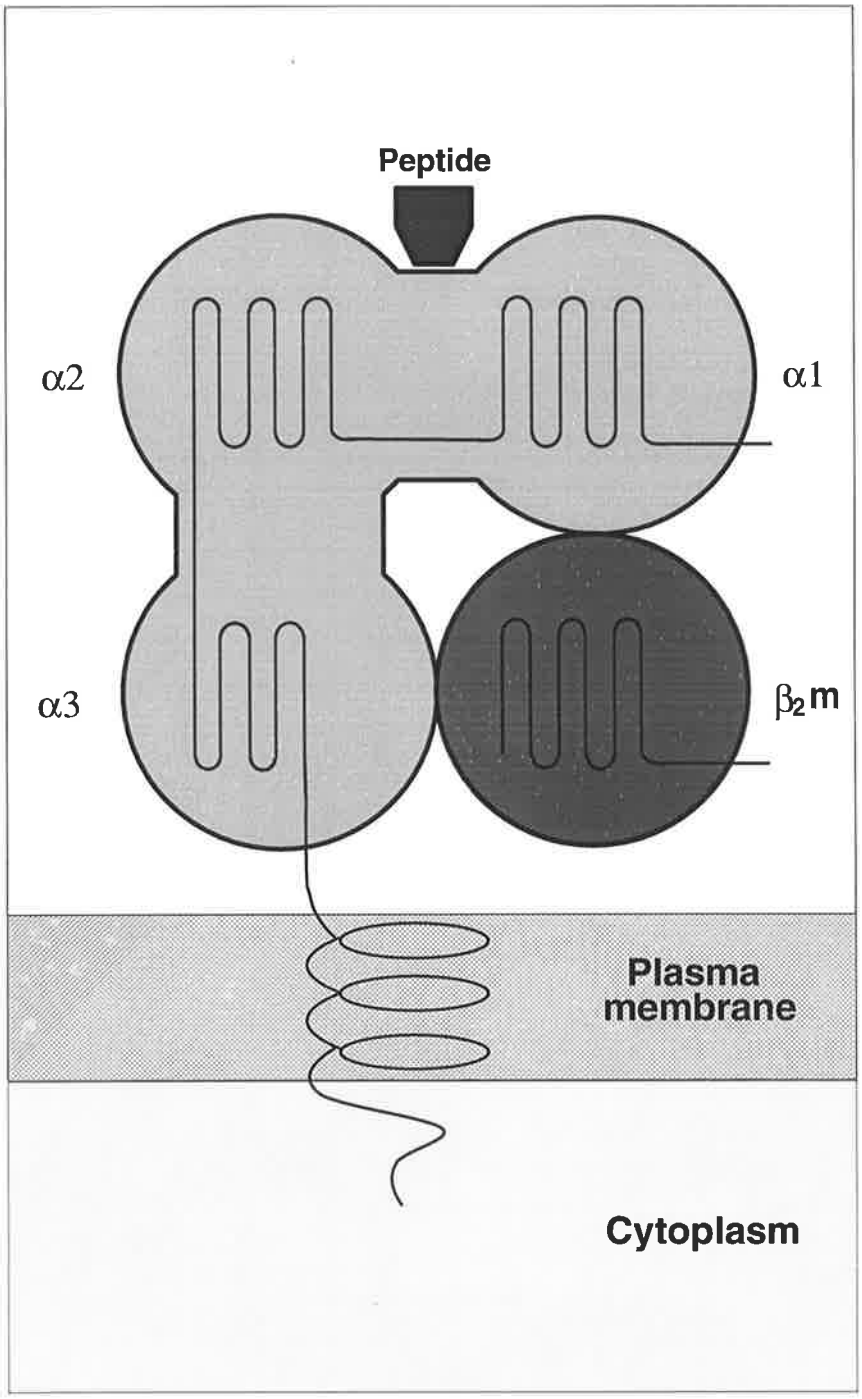
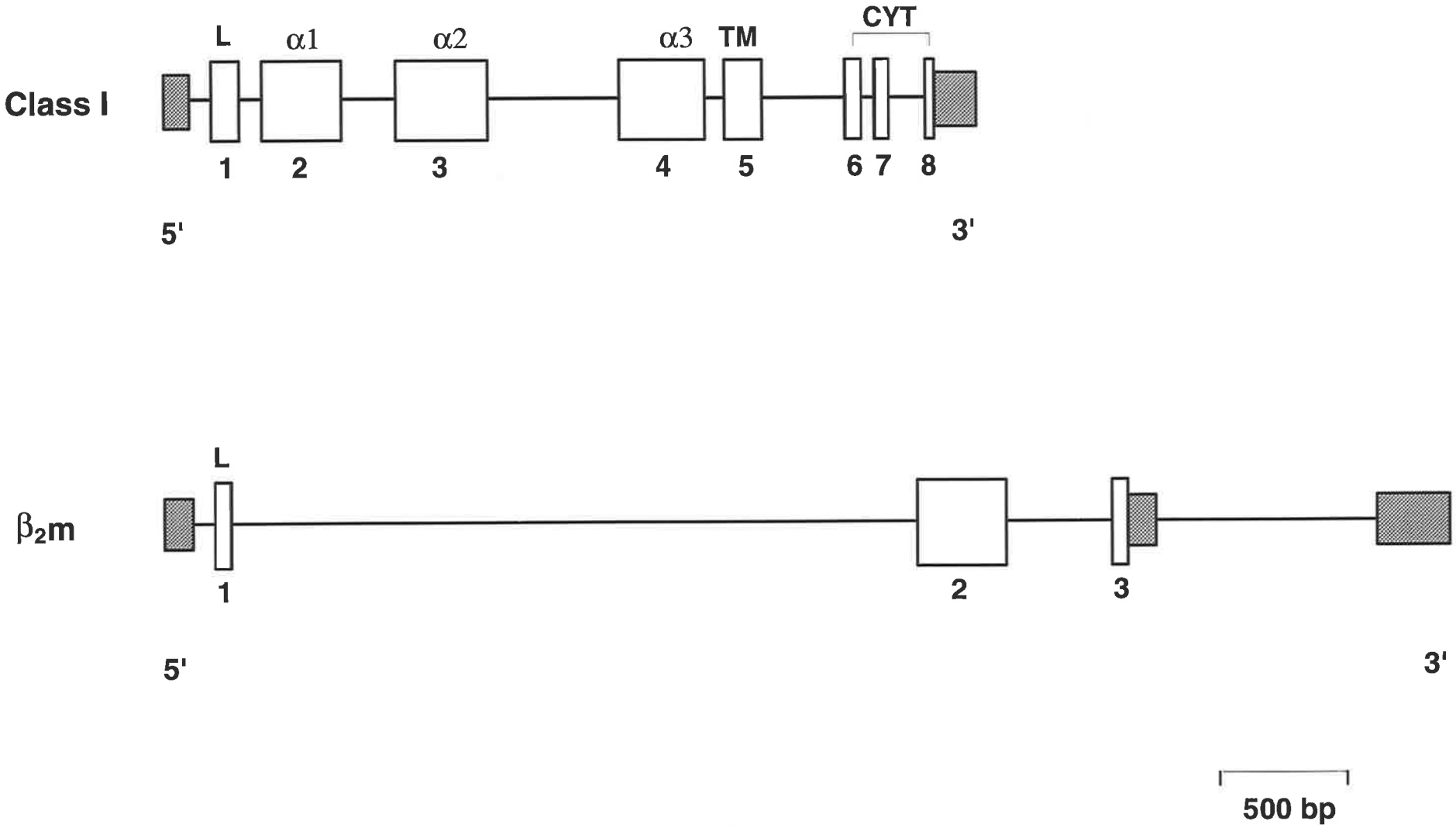

Peptide
α2
α1
α3
β2m
Plasma membrane
Cytoplasm

Figure 1.6

Map depicting the organization of MHC-Ia αC and β_2m introns (solid lines) and exons (numbered open boxes). The exons labelled α1, α2 and α3 encode the αC extracellular domains and those labelled L, TM and CYT encode the leader sequence, transmembrane and cytoplasmic domains, respectively. 5' and 3' untranslated regions are indicated by shaded boxes. Figure adapted from Flavell *et al.* (1986).


Class I
L
α1
α2
α3
TM
CYT
1
2
3
4
5
6
7
8
5'
3'
β2m
L
1
2
3
5'
3'
500 bp

of the β_2m protein and exon 3 encodes the remainder of the protein and a 3' untranslated region (Parnes *et al.,* 1981; Parnes & Seidman, 1982).

Crystallographic studies have shown that the two N-terminal αC domains (α1 and α2) fold together to form the sides of a peptide binding groove comprising 2 α helices, which sit on a platform of eight anti-parallel β-sheets (Bjorkman *et al.,* 1987 a). The peptide is bound in an extended conformation and side chains of the peptide are accomodated in specific pockets in the peptide groove (Madden *et al.,* 1991; Fremont *et al.,* 1992; Matsumura *et al.,* 1992). Below the antigen binding region, the α3 domain folds into an immunoglobulin-like domain that forms extensive contacts with β_2m (Michaelson, 1981). β_2m also makes extensive contacts with the β sheet floor of the α1 and α2 peptide binding region (Yokoyama & Nathenson, 1983; Bjorkman *et al.,* 1987 a; Saper *et al.,* 1991; Fremont *et al.,* 1992).

The differences between the products of different MHC-Ia alleles are virtually all accounted for by amino acid substitutions in the α1 and α2 domains of the αC (Hood *et al.,* 1983). The α3 domain is highly conserved (Steinmetz *et al.,* 1982) and β_2m is invariant (Michaelson *et al.,* 1980). Most of the amino acids that vary between MHC-Ia molecules are located at sites that alter the shape of the antigen binding groove (Bjorkman *et al.,* 1987 a, Garrett *et al.,* 1989) providing an understanding of how allelic variation among MHC-Ia genes directly influences the spectrum of peptides presented to $CD8^+$ T cells (Fremont

et al., 1992; Matsumura *et al.,* 1992).

1.6.2 Nature of antigenic peptides bound to MHC-Ia molecules

The first indication that $CD8^+$ T cells recognize peptides derived from viral proteins was the observation that peptides of <5kDa, derived from the enzymatic and chemical cleavage of Sendai virus proteins, stimulated Sendai virus specific $CD8^+$ T cell proliferation *in vitro* (Guertin & Fan, 1980). A series of studies by Townsend *et al.* (1984, 1985, 1986 a) resulted in the demonstration that cells incubated with synthetic peptides of 14 or more residues, corresponding to influenza virus nucleoprotein sequences, sensitized target cells for $CD8^+$ T cell recognition. Electron density mapping of a human MHC-Ia molecule (HLA-A2) showed that peptides co-localized with the HLA-A2 αC (Bjorkman *et al.,* 1987 a).

Amino acid sequencing of naturally processed peptides purified by immunoaffinity and acid elution methods has provided new insights into the nature of antigens bound to particular MHC-Ia molecules (Van Bleek & Nathenson, 1990; Falk *et al.,* 1991). For instance, Van Bleek and Nathenson (1990) recovered radiolabelled peptide from affinity-purified $H\text{-}2K^b$ molecules of cells infected with vesicular stomatitis virus (VSV). Sequencing showed the peptide to be an octamer, derived from the viral nucleocapsid protein. A synthetic peptide derived from this sequence was capable of sensitizing cells for recognition by VSV specific $H\text{-}2K^b$ restricted CTLs.

Important general characteristics of self peptides bound to MHC-Ia molecules were identified by Falk *et al.* (1991). Peptides eluted from mouse and human MHC-Ia molecules were pooled from HPLC fractions and sequenced. The peptides were 8-9 amino acids in length and predominant residues could be found in comparable positions in most peptides, giving rise to the concept of "anchor" amino acids important in promoting binding to a particular MHC-Ia allele. These findings were reinforced by a later study by Jardetzky *et al.* (1991) who sequenced HPLC purified peptides derived from HLA-B27 molecules and revealed all peptides consisted of nine residues, had an arginine at position 2 (numbering from the N-terminus) and a preference for a basic residue at the carboxyl-terminal ninth position.

Although several studies using a variety of techniques indicated that eight (K^b) or nine (D^b, K^d, L^d) amino acids was optimal for binding of peptides to MHC-Ia molecules (Reddehase *et al.,* 1989; Cerundolo *et al.,* 1991; Elliott *et al.,* 1991; Gould *et al.,* 1991; Pamer *et al.,* 1991; Romero *et al.,* 1991; Schulz *et al.,* 1991; Schumacher *et al.,* 1991; Deckhut *et al.,* 1992), the maximum length of peptides that can be accomodated is unknown. Naturally processed MHC-Ia bound peptides comprising 13 residues have been identified (Henderson *et al.,* 1992; Wei & Cresswell, 1992) and the significance of these observations needs to be established.

1.6.3 Assembly and transport of MHC-Ia molecules

1.6.3.1 Overview

MHC-Ia αCs and β_2m are synthesized with signal sequences, directing the nascent proteins to the ER. Following translocation to the ER, the signal sequences are removed and the proteins are glycosylated. The two subunits then fold and associate, forming an αC-β_2m dimer. Peptides are generated primarily in the cytoplasm and are subsequently transported into the ER by accessory molecules known as transporters associated with antigen processing (TAPs). In the ER, peptides associate with αC-β_2m dimers, giving rise to trimolecular complexes (αC-β_2m-peptide). Fully assembled trimolecular complexes are transported to the cell-surface, where they can be recognized by $CD8^+$ T cells. The steps involved in the biosynthesis, assembly and transport to the cell-surface of functional MHC-Ia trimolecular complexes are discussed in detail below.

1.6.3.2 The role of β_2-microglobulin

Assembly of MHC-Ia molecules was first analyzed using the monoclonal antibody W6/W2, which recognizes a determinant expressed on most human MHC-Ia molecules only when associated with β_2m, and a rabbit serum that reacts only with denatured αCs (Krangel *et al.,* 1979). The role of β_2m in promoting transport, conformational alteration and stabilization of αCs was demonstrated in a β_2m-deficient human cell line called Daudi (Arce-Gomez *et*

al., 1978). αCs expressed by Daudi cells are (i) malfolded, (ii) do not react with W6/W2, (iii) cannot be exported from the ER and (iv), are rapidly degraded (Kissonerghis *et al.,* 1980). Studies using β_2m-deficient mouse (RIE) cells have shown that transfection with MHC-Ia αC genes results in little or no detectable transport of αCs to the cell-surface (Allen *et al.,* 1986; Williams *et al.*, 1989). In these studies, any uncomplexed αC that did reach the cell-surface did so at very low efficiency and these surface molecules were not properly folded.

There is some evidence that suggests that αCs alone can be expressed on the cell-surface. For example, Hansen *et al.* (1988) transfected mouse splenocytes with a H-2L^d αC gene and showed that some cells could express free (ie. uncomplexed) αC on their surfaces. In addition, Bix and Raulet (1992) were able to show that very low levels of properly folded H-2D^b molecules could be expressed on the surface of T cells isolated from β_2m-deficient mice and on the surface of RIE cells transfected with H-2D^b αC. Although these data indicate that transport of αC-peptide complexes to the cell-surface can occur in the absence of β_2m, the very low level of cell-surface expression suggests that in normal cells such a mechanism of peptide presentation represents a minor pathway. Retention of malfolded or unassembled membrane or secretory proteins in the ER is a widely recognized phenomenon (Hurtley & Helenius, 1989; Rose & Doms, 1988). MHC-Ia αC retention in the ER has been reported in a diverse group of animal species, ranging from Xenopus oocytes

(Severinsson & Peterson, 1984) to humans (Serge *et al.,* 1981). The leakage of low levels of αCs to the cell-surface in β_2m-deficient cells possibly results from transient aquisition of a properly folded form of the molecule rather than evasion of the ER selection pathway (Hurtley & Helenius, 1989).

1.6.3.3 Generation of antigenic peptides

Peptide binding to MHC-Ia molecules is an essential requirement for the transport of virtually all MHC-Ia molecules from the ER to the cell-surface. Several lines of evidence suggest that the cell cytoplasm is the source of peptides that associate with MHC-Ia molecules.

(i) Peptides targeted to the cytoplasm using recombinant vaccinia virus expression vectors are presented to T cells by MHC-Ia molecules (Gould *et al.,* 1989; Whitton & Oldstone, 1989).

(ii) Addition of exogenous ovalbumin (OVA) to antigen presenting cells (APCs) *in vitro* does not result in the formation of OVA-MHC-Ia products. However, introduction of native OVA directly into the cytoplasm of APCs results in the association of OVA processed peptides with MHC-Ia products (Moore *et al.*, 1988).

(iii) Fusion of ubiquitin to the N-terminus of influenza nucleoprotein facilitates the presentation to T cells, by MHC-Ia molecules, of nucleoprotein derived peptides (Townsend *et al.,* 1988).

The generation of peptides presented on MHC-Ia molecules is still not fully understood. There has been speculation that a proteasome, which is the "catalytic core" of the ubiquitin-proteasome proteolytic pathway (Ciechanover, 1994), generates the peptides for MHC-Ia molecules (Goldberg & Rock, 1992; Monaco, 1992). This speculation came about following the discovery of 2 subunits of the multi-catalytic protein or proteasome; low molecular weight polypeptide (LMP)2 and LMP7 in mice (Brown *et al.,* 1991; Martinez & Monaco, 1991) and RING-12 and RING-10 in humans (Glynne *et al.,* 1991; Kelly *et al.,* 1991). These subunits are encoded in the MHC region in tandem with TAP-1 and TAP-2 genes (see section 1.6.3.4) and their expression is induced by γ-IFN (Monaco, 1992; Yang *et al.,* 1992 a; Brown *et al.,* 1993; Gaczynska *et al.,* 1993).

Proteasomes are abundant, ubiquitous and located in the cytoplasms and nuclei of all organisms (Orlowski, 1990). The end products of proteasome-mediated digestion are typically short peptides of different length (Dick *et al.,* 1991). However, although these two subunits form part of a LMP complex closely related to proteasomes (Brown *et al.,* 1991; Oritz-Navarrete *et al.,* 1991; Arnold *et al.,* 1992), there is no direct evidence linking them to the generation

of peptides for presentation by MHC-Ia molecules *in vivo*.

It has been shown that incorporation of LMP2 and LMP7 alters the peptidase cleavage specificity of proteasome complexes, in that they increase hydrolysis after basic and hydrophobic residues and decrease hydrolysis after acidic residues (Driscoll *et al.,* 1993; Gaczynska *et al.,* 1993; Aki *et al.,* 1994; Boes *et al.,* 1994). Proteasomes isolated from human lymphoblastoid (721.174 or T2) cells, which have large deletions in the MHC that encompass the genes encoding LMP2 and LMP7, exhibit a reduced ability to cleave peptides after basic and hydrophobic residues (Driscoll *et al.,* 1993; Gaczynska *et al.,* 1993) and an enhanced ability to cleave after acidic residues (Gaczynska *et al.,* 1993). The opposite effect is true of proteasomes isolated from cells treated with γ-IFN to induce LMP expression (Gaczynska *et al.,* 1993; Aki *et al.,* 1994). It is possible that the incorporation of LMPs in proteasomes favours the generation of peptides that can associate with MHC-Ia molecules, because most peptides that bind MHC-Ia molecules terminate almost exclusively with hydrophobic or basic residues (Falk & Rotzschke, 1993).

Dick *et al.* (1994) showed that proteasomes can generate MHC-Ia binding peptides from denatured OVA. In addition, Niedermann *et al.* (1995), using purified 20S proteasomes *in vitro*, demonstrated preferred cleavage sites directly adjacent to the amino- and carboxyl- terminal ends of the immunodominant epitope of chicken OVA. Variations in amino acid sequences

flanking these epitopes were shown to influence proteasomal cleavage patterns in parallel with the efficiency of their presentation by MHC-Ia molecules. These data suggest that proteasome-mediated proteolysis contributes to the hierarchy of epitopes presented by MHC-Ia molecules.

Mice with a targeted deletion in the gene encoding LMP7 were shown to have reduced MHC-Ia cell-surface expression and presented an endogenous antigen (HY) inefficiently. When peptides were added to splenocytes deficient in LMP7, wild-type MHC-Ia expression levels were restored (Fehling *et al.,* 1994). This *in vivo* study provided strong evidence for the involvement of LMP7 in the MHC-Ia presentation pathway. Although the exact function of LMP7 still remains unclear, the efficient restoration of normal MHC-Ia expression levels by antigenic peptides indicates a specific role for LMP7 in the peptide supply machinery. LMP7 may physically link the proteasome and the TAP transporter system to increase the efficiency of peptide translocation. Alternatively, LMP7 may actively participate in the generation of antigenic peptides by altering the specificity of the proteasome. These potential functions require further investigation.

Van Kaer *et al.* (1994) demonstrated that LMP2-deficient mice had an altered peptidase activity. These alterations resembled the pattern seen previously in MHC-Ia-deficient cell lines lacking the genes encoding LMP2 and LMP7 (Driscoll *et al.,* 1993; Gaczynska *et al.,* 1994) and were opposite to the effects

seen following induction of LMPs by γ-IFN (Gaczynska *et al.*, 1993; Aki *et al.*, 1994). Further, LMP2-deficient mice also had reduced levels of $CD8^+$ T cells compared with wild-type mice. However, there was no significant effect on cell-surface MHC-Ia expression (Van Kaer *et al.*, 1994). These data indicate that LMP2 and (and possibly also LMP7), induce alterations in the subunit organization of proteasomes, which may modify the proteolytic capabilities of the proteasome complex in a manner that is advantageous for presentation of MHC-Ia restricted antigens to the immune system.

Rock *et al.* (1994) have shown that inhibitors of proteasome chymotryptic activity block immune presentation of OVA by preventing its proteolytic degradation. Thus, it appears that proteasomes catalyse the degradation of the vast majority of cell proteins and generate peptides that can be presented by MHC-Ia molecules. However, whether proteasome products are optimal peptides for association with MHC-Ia molecules remains unclear (Dick *et al.*, 1994).

Several studies have shown that MHC encoded proteasome subunits are not essential for MHC-Ia antigen presentation. For example, LMP-deficient cell lines can generate peptides that associate with MHC-Ia molecules in the ER (Arnold *et al.*, 1992; Momburg *et al.*, 1992; Yewdell *et al.*, 1994; Zhou *et al.*, 1994).

MHC-Ia associated peptides considerably longer than 8-10 amino acids have been found in cells (Udaka *et al.,* 1993; Joyce *et al.,* 1994; Urban *et al.,* 1994). It is not known with certainty whether these peptides (up to 33 residues) are intermediates in the antigen presentation pathway, but two studies have provided evidence consistent with the notion that cleavage of longer precursors to final peptide products can occur in the ER (Eisenlohr *et al.,* 1992; Malarkannan *et al.,* 1995). Recently, Buchholz *et al.* (1995) demonstrated that residues flanking optimal MHC-binding peptides were cleaved from endogenously synthesized precursors, indicating that they were processed. However, precursors that matched the optimal length of MHC-binding peptides were presented on the cell-surface with comparable efficiency. These data suggest that cleavage of flanking residues is not essential for intracellular assembly or cell-surface expression of MHC-Ia-peptide complexes and that precursor peptide cleavage is an independent step in the antigen presentation pathway.

1.6.3.4 Transport of peptides into the endoplasmic reticulum

Mechanisms are required to transport peptides into the lumen of the ER because peptides are generated in the cytoplasm whereas MHC-Ia molecules are cotranslationally inserted into the ER. It appears that cytoplasmic peptides can use at least two different mechanisms to enter the ER.

Transporters associated with antigen processing (TAP): There is substantial evidence that a heterodimeric complex encoded within the MHC class II region and called the transporter associated with antigen processing (TAP) facilitates entry of peptides into the ER. The components of this complex, known as TAP-1 and TAP-2, share substantial homologies with the ATP-binding cassette (ABC) family of ATP-dependent transporter proteins. These genes were originally referred to as HAM-1 and HAM-2 in mice (Monaco *et al.,* 1990), mtp-1 and mtp-2 in rats (Deverson *et al.,* 1990) and PSF-1 and PSF-2 (Spies *et al.,* 1990) or RING-4 and RING-11 in humans (Trowsdale *et al.,* 1990; Powis *et al.,* 1992 a). Their products have a molecular weight of 75kDa and are induced by γ-IFN. The ABC family of membrane translocators includes the cystic fibrosis chloride channel (Riordan *et al.,* 1989), the yeast STE6 protein (McGrath & Varshavsky, 1989), the multidrug resistance p-glycoprotein (Gros *et al.,* 1986) and the *Salmonella typhimurium* oligopeptide transporter (Hiles *et al.,* 1987). These proteins all have a hydrophobic domain that is thought to consist of 6-8 membrane-spanning α helices, followed by a large cytoplasmically located domain that contains an ATP-binding cassette. It was speculated that TAP-1 and TAP-2 gene products may transport peptides from the cytoplasm to the ER (Deverson *et al.,* 1990; Monaco *et al.,* 1990; Spies *et al.,* 1990; Trowsdale *et al.,* 1990; Bahram *et al.,* 1991) because other members of the ABC protein family, including STE6 and oppDF, could transport peptides (Higgins *et al.,* 1990).

TAP-1 and TAP-2 have been shown to physically associate (Kelly *et al.,* 1992; Spies *et al.,* 1992). Further, immunohistochemical studies have shown that TAP complexes are confined to the ER and *cis*-golgi membranes (Kleijmeer *et al.,* 1992), consistent with a role in peptide transport into the ER. Recently, it was shown that TAP complexes bind to αC-β_2m dimers (Androlewicz *et al.,* 1994; Suh *et al.,* 1994; Ortmann *et al.,* 1994). Association of the TAP complexes with MHC-Ia molecules may promote efficient capture of antigenic peptides.

Genetic analysis of TAP-deficient cell lines suggests that in the absence of TAP-1 or TAP-2, αC-β_2m complexes are unstable and MHC-Ia dependent antigen presentation is lost. Further, transfection studies using TAP-deficient cell lines showed that TAP-1 and TAP-2 are required to restore antigen presentation (DeMars *et al.,* 1985; Salter *et al.,* 1985; Salter & Cresswell, 1986; Cerundolo *et al.,* 1990; Powis *et al.,* 1991; Spies & DeMars, 1991; Arnold *et al.,* 1992; Attaya *et al.,* 1992; Kelly *et al.,* 1992; Momburg *et al.,* 1992; Spies *et al.,* 1992; Yang *et al.,* 1992 b).

Several groups have provided evidence suggesting that TAP-mediated transport of peptides into the ER is ATP-dependent (Androlewicz *et al.,* 1993; Neefjes *et al.,* 1993; Shepherd *et al.,* 1993). However, studies of peptide translocation in purified microsomes (Levy *et al.,* 1991; Koppelman, *et al.,* 1992) and in minigene constructs encoding MHC-Ia-specific peptides (Anderson *et al.,* 1991;

Zweerink *et al.,* 1993), have shown that peptides could be translocated in an ATP-independent manner. Therefore, it is not clear whether ATP is essential for efficient peptide transport.

Direct assays of TAP-mediated peptide transport into the ER, utilizing either intact microsomes or permeabilized cells, have revealed that peptides ranging in length from 8-15 amino acids are transported and some sequence preferences have been shown (Neefjes *et al.,* 1993; Shepherd *et al.,* 1993; Androlewicz *et al.,* 1993; Heemels *et al.,* 1993; Momburg *et al.,* 1994 a, b; Schumacher *et al.,* 1994). Shorter and longer peptides are translocated with decreased efficiency (Momburg *et al.,* 1994 b). These studies suggest that TAP complexes selectively and preferentially translocate peptides of appropriate length for binding to MHC-Ia molecules.

A potential role for TAP-1 in peptide transport has been demonstrated *in vivo.* First, mice deficient in TAP-1 are defective in the stable assembly and intracellular transport of MHC-Ia molecules and have reduced levels of cell-surface MHC-Ia expression. Second, addition of peptide to spleen cells derived from TAP-1 deficient mice was shown to stabilize αC-β_2m dimers (Van Kaer *et al.*, 1992).

Signal recognition particle (SRP)-dependent pathway: Peptides fused to a hydrophobic ER insertion signal sequence can be delivered into the ER of TAP-deficient cells and presented in association with MHC-Ia molecules at the cell-surface (Anderson *et al.,* 1991; Eisenlohr *et al.,* 1992). In addition, MHC-Ia molecules in TAP-deficient T2 cells bind predominantly to peptides derived from N-terminal signal sequences of endogenous proteins (Henderson *et al.,* 1992; Wei & Cresswell, 1992). These peptides also bind to MHC-Ia molecules expressed by normal cells (Henderson *et al.,* 1992), although it is important to note that they form only a minor component of the peptide repertoire. Further, Bacik *et al.* (1994) demonstrated that ER insertion sequences that enhance presentation of endogenously synthesized peptides to MHC-Ia molecules must be located at the amino rather than the carboxyl-terminus of the peptide. Thus, the SRP-dependent pathway of peptide delivery provides an alternative mechanism by which cytosolyic peptides gain access to MHC-Ia molecules, bypassing the TAP-dependence of peptide presentation. However, the significance of the SRP-dependent pathway in peptide transport is not clear because peptides bound by MHC-Ia molecules do not require signal sequences (Townsend *et al.,* 1985, 1986 b).

1.6.3.5 Accessory molecules involved in MHC-Ia assembly

αCs and β_2m are synthesized with signal sequences that target them to the ER. These classical N-terminal signal sequences are removed once the molecules have been cotranslationally translocated into the ER (Dobberstein *et al.,* 1979; Ploegh *et al.,* 1979; Owen *et al.,* 1980). The αC associates rapidly with an ER protein called p88 (Degen & Williams, 1991; Wada *et al.,* 1991; Degen *et al.,* 1992). The αC-p88 complex was shown to exist transiently and the length of association correlated directly with the rate of ER to golgi transport of each αC. Based on immunological criteria, p88 has been identified as calnexin (Ahluwalia *et al.,* 1992; Galvin *et al.,* 1992), a molecular chaperone known to bind transiently to a variety of membrane proteins in the lumen of the ER (David *et al.,* 1992; Hochstenbach *et al.,* 1992; Ou *et al.,* 1993).

In β_2m-deficient cells, calnexin remains attached to αCs, suggesting that a function of calnexin is to retain αCs in the ER until they associate with β_2m and peptide (Degen *et al.,* 1992). Margolese *et al.* (1993) suggested that the αC transmembrane domain and three flanking amino acids interact with calnexin, based on the observation that no interaction could be detected between calnexin and a glycosyl phosphatidylinositol (GPI) anchored MHC-Ia molecules, which lacked the cytoplasmic and transmembrane domains.

Recently, Jackson *et al.* (1994) expressed αC with or without β_2m and calnexin in *Drosophila melanogaster* cells. Calnexin was shown to retard the intracellular transport both of peptide-deficient αC-β_2m dimers and free αC. Calnexin also slowed the intracellular degradation of free αC. The ability of calnexin to protect and retain incompletely assembled MHC-Ia complexes is likely to contribute to the efficiency of intracellular formation of MHC-Ia complexes.

Calnexin has a carboxyl-terminal amino acid motif that has been shown to be responsible for its retention in the ER (Rajagopalan *et al.,* 1994). Deletion of this motif by truncation of the calnexin cytoplasmic tail resulted in calnexin exiting the ER and localizing in the golgi. Rajagopalan & Brenner (1994) showed that in β_2m-deficient FO-1 cells, cytoplasmic tail deletion mutants of calnexin leave the ER and progress to the cell-surface with free αCs. These data suggest that calnexin determines the intracellular localization of associated MHC-Ia molecules, strengthening the hypothesis that it is responsible for retention of partially assembled MHC-Ia complexes in the ER.

For unknown reasons, different MHC-Ia molecules are exported from the ER at different rates (Williams *et al.,* 1985; Degen & Williams, 1991). If the release of calnexin from αCs is triggered by conformational changes caused by peptide binding, then transport rates may reflect the availability of high affinity peptides that can bind to each αC. Alternatively, calnexin may have different

affinities for different αCs. Single amino acid substitutions in αCs have been shown to cause significant reductions in their transport rates (Miyazaki *et al.*, 1986; Williams *et al.*, 1988). In most cases mutant αCs have an altered conformation, implying that correct conformation is necessary to attain a transport competent state (Rose & Doms, 1988).

1.6.3.6 Summary of MHC-Ia assembly and transport

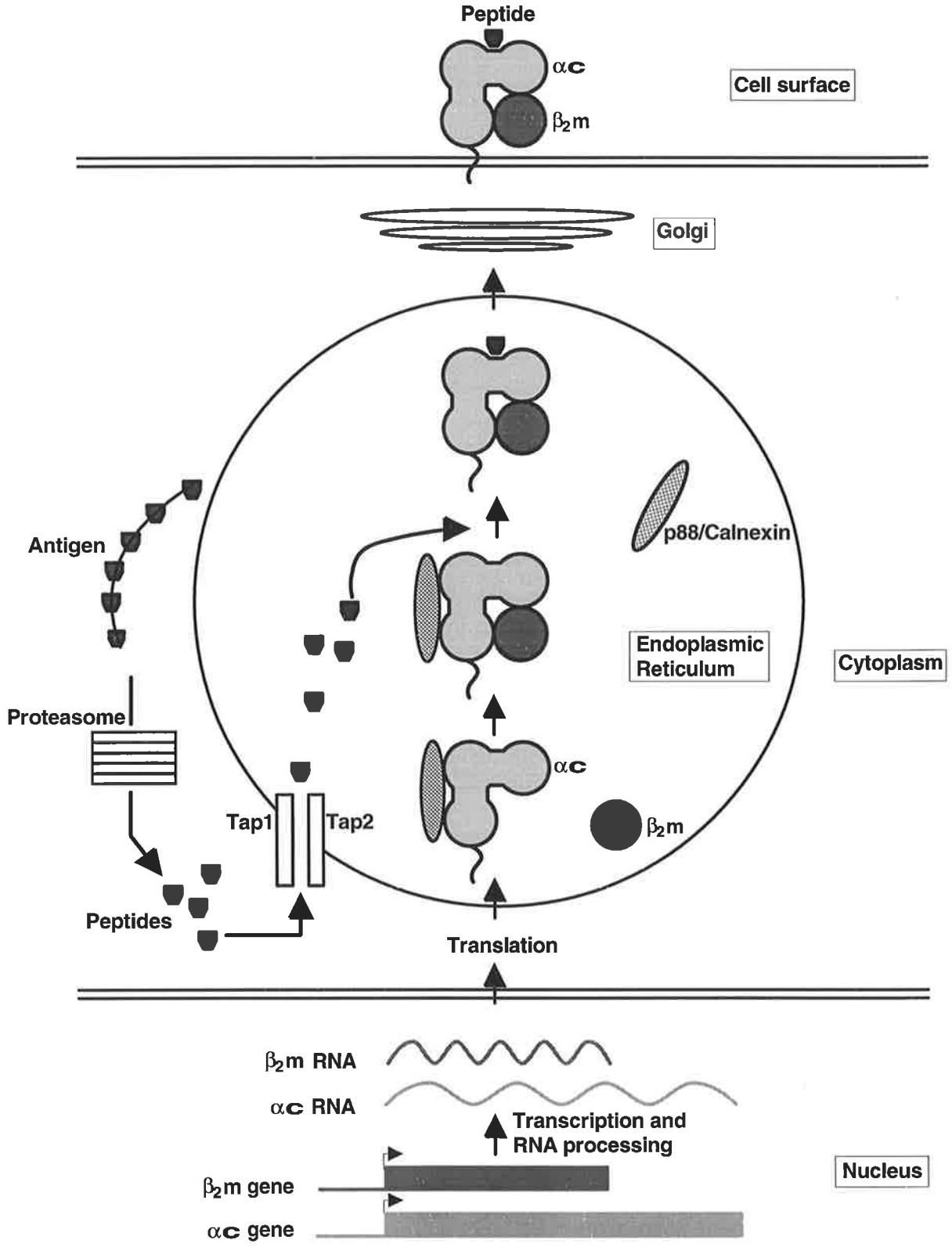
Current understanding of the MHC-Ia assembly pathway (Figure 1.7) can be summarized as follows. αCs and β_2m are targeted to the ER via classical N-terminal signal sequences. After entry into the ER, the signal sequences are removed and the molecules associate to form heterodimers, which are maintained in the ER by association with calnexin. Peptides are generated from nascent cytoplasmic proteins by proteasomal digestion and transported by TAPs into the ER where they complex with αC-β_2m dimers. Release of calnexin from αCs follows binding of peptide to the αC-β_2m dimer. Finally, each trimolecular complex comprising an αC, β_2m and a peptide is transported along the exocytic pathway to the cell-surface.

1.6.4 Other molecules involved in T cell-MHC-Ia recognition

Antigenic peptides are recognized in the context of MHC-Ia molecules by antigen-specific T cell receptors (TCR) on the surfaces of T cells. In addition, accessory surface T cell antigens CD8, CD2 and the lymphocyte functional antigen (LFA)-1 play an essential role in CTL adhesion to target cells (Figure

Figure 1.7

Schematic representation of the biosynthesis and assembly of surface MHC-Ia molecules. The MHC-Ia αC and β_2m genes are transcribed and processed in the nucleus and subsequently co-translationally translocated into the endoplasmic reticulum (ER), where they dimerize. Calnexin (p88) binds to, and stabilizes free αC and αC-β_2m dimers in the ER. Intracellular proteins are degraded into antigenic peptides in the cytoplasm by proteasomal digestion. Peptides are transported into the ER by the TAP complex. Peptides bind to αC-β_2m dimers, facilitating displacement of calnexin. The ternary MHC-Ia complex (αC, β_2m and peptide) is subsequently transported via the golgi to the cell-surface. Figure adapted from Jackson & Peterson (1993).


Peptide
αc
β2m
Cell surface
Golgi
Antigen
p88/Calnexin
Endoplasmic Reticulum
Cytoplasm
Proteasome
αc
Tap1
Tap2
β2m
Peptides
Translation
β2m RNA
αc RNA
Transcription and RNA processing
Nucleus
β2m gene
αc gene

1.8) (Berke, 1980; Dustin & Springer, 1991; O'Rourke *et al.,* 1991; Martz, 1993; O'Rourke & Mescher, 1993).

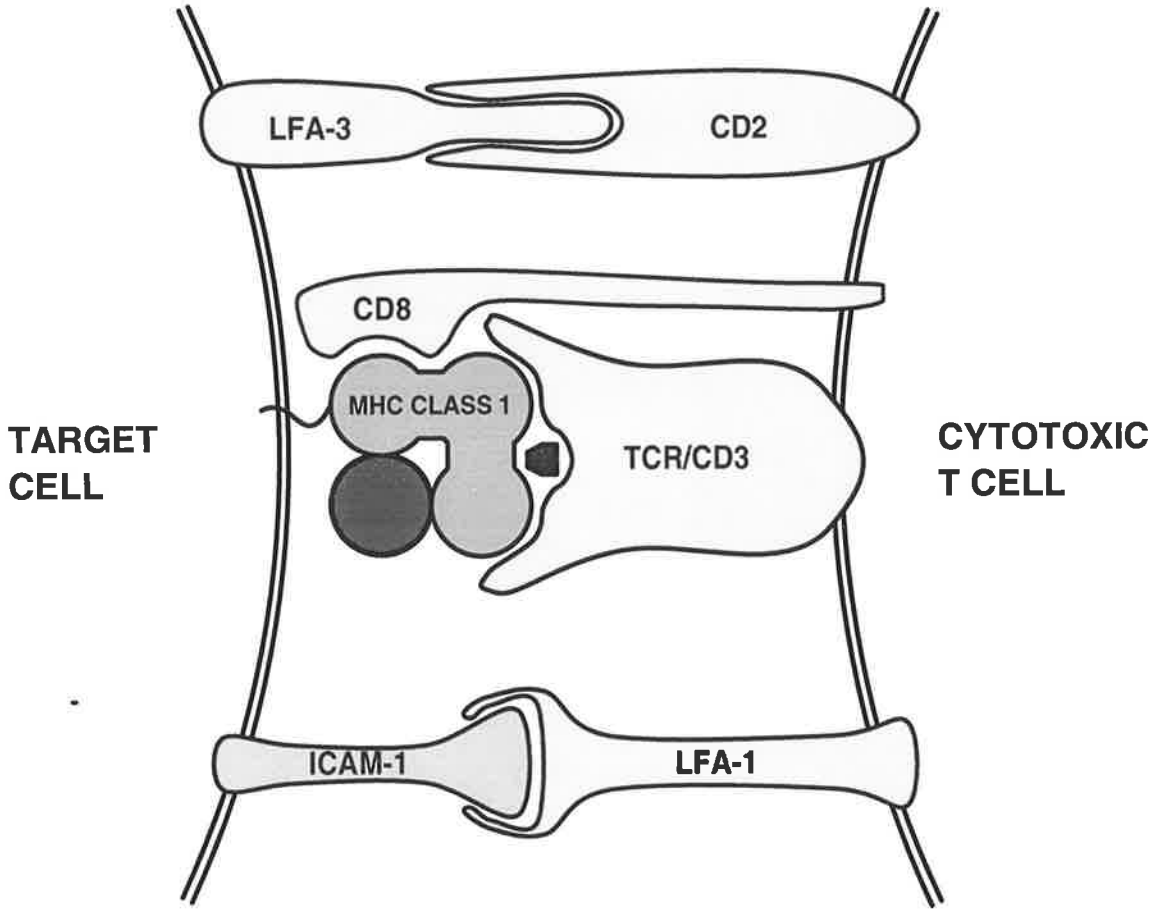
TCR: Antigen-specificity is conferred to the TCR by two subunits, each comprising variable and constant domains (Marrack & Kappler, 1986; Brenner *et al.,* 1988). These two subunits, termed α and β or γ and δ, depending on the class of TCR, are always associated with CD3 (Clevers *et al.,* 1988; Weissman *et al.,* 1989). This single structural unit is termed the TCR. The TCR recognizes bound peptide and surrounding polymorphic residues in the membrane distal domains of the MHC-Ia molecule (Bjorkman *et al.,* 1987 b).

CD8: CD8 binds to determinants on MHC-Ia molecules (Swain, 1983; Bierer *et al.,* 1989). The sites on the MHC-Ia molecule recognized by CD8 and the TCR appear distinct because CD8 binds to monomorphic residues in a membrane proximal domain of MHC-Ia molecules (Potter *et al.,* 1989; Salter *et al.,* 1989). CD8 and the TCR diffuse independently in the T cell membrane, until they are brought together by recognition of the same MHC-Ia-peptide complex (Springer, 1990).

LFA-1 and CD2: Screening of monoclonal antibodies for their ability to inhibit T cell mediated lysis led to the identification of two distinct molecules on the surfaces of CTLs; LFA-1 and LFA-2 (now called CD2). Studies have shown that LFA-1 and CD2 interact with target cell determinants called intercellular

Figure 1.8

Schematic representation of interactions involved in cytotoxic T lymphocyte (CTL) recognition of a target cell. The antigenic peptide derived from the target cell is presented to the T cell receptor complex (TCR/CD3) in association with an MHC-Ia molecule. CD8 binds to the membrane proximal $\alpha 3$ domain of the MHC-Ia molecule and stabilizes the target cell-CTL interaction. Stabilization is also conferred by ICAM-1 binding to LFA-1 through its N-terminal immunoglobulin (Ig) domain and LFA-3 binding to the N-terminal Ig domain of CD2. Figure adapted from Lee (1994).


LFA-3
CD2
CD8
MHC CLASS 1
TCR/CD3
ICAM-1
LFA-1
TARGET CELL
CYTOTOXIC T CELL

adhesion molecule (ICAM)-1 and LFA-3, respectively, during T cell recognition (Shaw *et al.*, 1986; Spits *et al.*, 1986; Shaw & Luce, 1987). The contribution of these cell-surface adhesion molecules to TCR-MHC-Ia-peptide interaction was demonstrated by the following observations: (i) monoclonal antibodies to any of these surface molecules could inhibit T cell mediated killing and (ii), the TCR alone had an extremely low affinity for MHC-Ia-peptide complexes (Matsui *et al.,* 1991; Weber *et al.,* 1992; Davis & Chien, 1993; Dal Porto *et al.,* 1993).

It has been proposed that antigen-independent adhesion is the initiating event in T cell recognition (Shaw *et al.,* 1986; Spits *et al.,* 1986). The LFA-1-ICAM-1 and CD2-LFA-3 molecular interactions play a crucial role in the establishment of antigen-independent contact between T cells and potential target cells (Shaw *et al.,* 1986; Spits *et al.,* 1986; Shaw & Luce, 1987). These interactions may be important to (i) overcome the mutual repulsion between cells, (ii) intimately oppose the plasma membranes of interacting cells or (iii), to allow for time during which membrane diffusion could enable the TCR to contact the MHC-Ia-peptide complex (Makagoba *et al.,* 1988). The strength and nature of the initial adhesion event is dependent on many factors, including the surface molecules expressed by each cell and the state of activation of the T cell (Rothlein *et al.*, 1986; Shaw & Luce, 1987). At this stage, cell to cell adhesion does not involve antigen recognition and is of low affinity.

Binding of CD2 to LFA-3 may transduce a signal that augments or synergizes with signals from the TCR to trigger T cell stimulation (Hunig *et al.,* 1987; Bierer *et al.,* 1989). This stimulation is dependent on the presence of the TCR (Tiefenthaler *et al.,* 1987; Bockenstedt *et al.,* 1988; Dustin *et al.,* 1989; Moingeon *et al.,* 1989). Binding of MHC-Ia-peptide complexes to TCRs strengthens the bond between LFA-1 and ICAM-1, which in turn enhances the strength of binding between a CTL and its target cell (Spits *et al.*, 1986; Dustin & Springer, 1989). Binding between LFA-1 and ICAM-1 is not sufficient to maintain cell to cell adhesion without a concomitant antigen recognition (Yannelli *et al.,* 1986; Reynolds & Ortaldo, 1990). The LFA-1-ICAM-1 interaction is involved in signal transduction, synergizing with signals from the TCR to activate T cells (Pircher *et al.,* 1986; Van Noesel *et al.,* 1988; Van Seventer *et al.,* 1990; Kuhlman *et al.,* 1991).

It is plausible that down-regulation of cell-surface adhesion molecule expression could render target cells less susceptible to recognition by CTLs. Gregory *et al.* (1988) reported down-regulation of LFA-3 and ICAM-1 in EBV positive Burkitt's lymphoma cells, and suggested that this represented a strategy by which tumour cells could escape virus-specific T cell surveillance. It has been shown that unlike other CNS cells, neurons are resistant to lysis by alloantigenic CTLs (Bartlett *et al.,* 1989; Keane *et al.,* 1992). In this respect, neuroblastoma cell lines constitutively lack ICAM-1 expression (Birdsall, 1991), which cannot be restored by exposure to γ-IFN. This has led to the

hypothesis that neurons may evade CTL mediated lysis by down-regulating ICAM-1 expression (White *et al.,* 1994).

1.7 Regulation of MHC-Ia expression in the nervous system

Neurons and cells of neuronal origin express unusually low levels of MHC-Ia molecules (Berah *et al.,* 1970; Edidin, 1972; Schachner & Seidman, 1973; Schachner & Hammerling, 1974; Vitetta & Capra, 1978; Williams *et al.,* 1980; Daar *et al.,* 1984; Wong *et al.,* 1984, 1985; Bartlett *et al.,* 1989; Joly *et al.,* 1991). Neurons and many neuronally derived cell lines tightly regulate MHC-Ia expression at the level of αC transcription (Joly *et al.*, 1991; Massa *et al.*, 1993). There is some evidence that failure to transcribe αCs may render virally infected neurons resistant to CTL mediated lysis. For instance, the retrovirally transformed cell line (OBL21) expresses barely detectable levels of MHC-Ia molecules and lymphocytic choriomeningitis virus (LCMV) infected OBL21 cells are resistant to lysis by LCMV-specific CTLs (Joly *et al.,* 1991). Transfection of OBL21 cells with an αC expression vector restored functional, cell-surface MHC-Ia antigen expression and susceptibility to CTLs (Joly *et al.,* 1991).

resistant?

In the CNS, neurons are resilient to induction of MHC-Ia antigens by viral infections or treatment with cytokines (Momburg *et al.,* 1986; Mauerhoff *et al.,* 1988). In contrast, glial cells can be readily induced to express MHC-Ia

molecules *in vitro* (Wong *et al.,* 1984; Skias *et al.,* 1987; Lavi *et al.,* 1988; Liu *et al.,* 1989) or *in vivo* (Massa *et al.,* 1986; Suzumura *et al.,* 1986; Lindsley *et al.,* 1988; Weinstein *et al.,* 1990; Gogate *et al.,* 1991) using various treatments including γ-IFN. On the basis of these observations Massa *et al.* (1993) studied the mechanisms of cell-type specific MHC-Ia gene regulation in γ-IFN treated primary cultures of astrocytes, oligodendrocytes and neurons. γ-IFN treatment induced MHC-Ia expression in astrocytes and oligodendrocytes but not in neurons. Differential regulation of MHC-Ia expression in γ-IFN treated neurons and glia was linked to the absence in neurons of a nuclear factor that binds to the MHC-Ia regulatory element (MHC-CRE) region I and the IFN-response consensus sequence (ICS).

Several studies have suggested that cells of neuronal origin are not as resistant to γ-IFN induction of MHC-Ia molecules as previously thought. For instance, γ-IFN treated IMR-5 (human neuroblastoma) cells upregulate the expression of a MHC-CRE region I binding protein and express MHC-Ia molecules (Drew *et al.,* 1993). In addition, γ-IFN treatment has been shown to upregulate MHC-Ia expression in neurons *in vivo*. For example, injection of γ-IFN or β-IFN into mouse brains induced MHC-Ia expression in neurons (Wong *et al.,* 1985; Fueta *et al.,* 1989) and γ-IFN treated cultured rat hippocampal neurons could transcribe MHC-Ia genes and express cell-surface MHC-Ia molecules (Neumann *et al.,* 1995). In the latter study, only electrically silent cells, presumed to represent damaged neurons, responded to γ-IFN. Furthermore,

viral infection has been shown to induce MHC-Ia expression *in vivo*. Primary sensory neurons in mouse sensory nerve ganglia upregulate MHC-Ia in response to HSV infection (Pereira *et al.*, 1994).

In addition to constraints on transcription of αC genes, failure to transcribe TAP-1 and TAP-2 mRNAs may contribute to the absence of MHC-Ia complexes on the surfaces of neurons (Joly & Oldstone, 1992). The evidence for this proposal is derived from the study of OBLψ2D^b cells, which express D^b molecules as a result of transfection of D^b αC into OBL21 cells, using a retroviral vector. OBLψ2D^b cells fail to provide MHC-Ia molecules with antigenic peptides and have undetectable levels of TAP-1 and TAP-2 mRNA. LCMV infected OBLψ2D^b cells are not lysed by LCMV-specific CTLs. γ-IFN treatment of OBLψ2D^b cells upregulated TAP-1 and TAP-2 expression and restored expression of cell-surface MHC-Ia molecules associated with antigenic peptides and susceptibility to antiviral CTLs (Joly & Oldstone, 1992). This result conflicts with the previous study of Joly *et al.* (1991), discussed above. Interestingly, TAP-1 and TAP-2 deficiency has not been reported in neurons *in vivo*.

In conclusion, although several studies have suggested that neurons and cells of neuronal origin are deficient in MHC-Ia expression, particularly at the level of αC transcription, there is emerging evidence that under certain conditions MHC-Ia expression can be restored.

1.8 Modulation of MHC-Ia expression by HSV infection

HSV is one of several viruses that has been shown to interfere with MHC-Ia expression (Jennings *et al.*, 1985; Hill *et al.*, 1994; York *et al.*, 1994; Hill *et al.*, 1995; Fruh *et al.*, 1995), presumably as a strategy for avoiding $CD8^+$ cell mediated lysis of infected cells. It has been postulated that interference with MHC-Ia expression might promote virus persistence in the host, by rendering infected cells invisible to a crucial arm of the immune response.

HSV infected human fibroblasts are resistant to attack by HSV-specific CTLs at an early stage of infection, prior to shut off of host cell protein synthesis by the VHS protein (Posavad & Rosenthal, 1992; Koelle *et al.*, 1993). Presentation of viral antigens to CTLs is defective as early as 2 hours after infection (Posavad & Rosenthal, 1992; Koelle *et al.*, 1993). CTL mediated cytolysis can be restored by addition of appropriate peptide antigens to the infected fibroblasts (Koelle *et al.*, 1993), suggesting that fibroblasts are not inherently resistant to CTL lysis.

The mechanism by which HSV prevents CTLs from recognizing infected fibroblasts has recently become apparent. MHC-Ia molecules have been shown to be retained in the ER of HSV infected human fibroblasts instead of being transported to the cell-surface (Hill *et al.*, 1994; York *et al.*, 1994). Transport of MHC-Ia molecules to the cell-surface is blocked by a HSV-1 IE gene

product, ICP47, which binds to the TAP complex, preventing peptide translocation into the ER (Hill *et al.,* 1995; Fruh *et al.,* 1995). Inhibition of peptide translocation by a virally encoded protein indicates a potentially novel mechanism for evasion of the immune response.

1.9 Gene delivery into the nervous system

Delivery of foreign genes into cells of the nervous system, particularly neurons, is a useful tool for studying the molecular aspects of development, function and survival of neural cells in the nervous system. There are three commonly used approaches to introduce DNA into cells; DNA transfection, use of retrovirus vectors and construction of transgenic animals.

DNA transfection: A variety of DNA transfection methods have been utilized, including co-precipitation of DNA with calcium phosphate (Graham & Van der Eb, 1973), treatment of recipient cells with high molecular weight cations such as dextran (Sompayrac & Danna, 1981), electroporation (Potter *et al.,* 1984) and micro-injection (Noda *et al.,* 1983). Transfection is effective for dividing cells but is often inefficient when used on post-mitotic, differentiated cells such as neurons (Geller, 1991; Leib & Olivo, 1993).

Retrovirus vectors: Retrovirus vectors (Gilboa *et al.,* 1986) have been used successfully for tracing cell lineage and for studying neuronal differentiation (Sanes *et al.,* 1983; Price *et al.,* 1987; Turner & Cepoka, 1987), protein synthesis and protein processing (Edwards *et al.,* 1986). A major advantage of retroviruses is their ability to integrate into host chromosomes. However, retroviruses require at least one mitotic cycle to be stably integrated (Cone & Mulligan, 1984) and are therefore ineffective as vectors for gene transfer into non-dividing cells (Miller *et al.,* 1990).

Transgenic animals: The many uses of transgenic animals (Palmiter & Brinster, 1985) include the study of gene regulation (Swanson *et al.,* 1985) and oncogene function (Palmiter *et al.,* 1985), the correction of genetic disorders (Readhead *et al.,* 1987) and the ablation of cells during development (Palmiter *et al.,* 1987). In addition, use of cell-specific promoters has enabled foreign gene expression to be studied in specific cell types (Townes *et al.*, 1985; Goring *et al.*, 1987; Kollias *et al.*, 1987; Readhead *et al.*, 1987; Forss-Petter *et al.*, 1990; Rall *et al.*, 1994, 1995).

Recently, HSV has been promoted as a vector for the introduction of DNA into fully differentiated neurons. The following factors facilitate its use as a vector.

(i) The DNA sequences of the HSV-1 and HSV-2 genomes are known (Davison & Wilkie, 1981; Hodgman & Minson, 1986; McGeoch *et al.*, 1987, 1988, 1991; Perry & McGeoch, 1988).

(ii) The 152kb genome can accomodate insertion of large genes (Longnecker *et al.*, 1988).

(iii) Many regions of the HSV genome, including the LAT locus, can be disrupted or eliminated without impairment of virus spread to neurons (Breakefield & DeLuca, 1991).

(iv) HSV has a wide host range (Roizman, 1990).

(v) HSV spreads to the nervous system and preferentially infects neurons (Wildy, 1967).

1.9.1 Types of HSV vectors

Two types of HSV vectors have been utilized for gene transfer, namely recombinant viruses and amplicons.

Recombinant viruses: The first step in the construction of a recombinant HSV usually involves cloning of a targeted region of HSV DNA into a plasmid vector to allow it's manipulation *in vitro*. The genetically engineered plasmid

containing the desired foreign gene flanked by HSV DNA sequences is then transfected into cultured cells along with viral DNA, generating recombinant viruses by homologous recombination. Recombination is favoured by using an excess of plasmid to viral DNA and exactly maintaining homologous viral sequences. Recombinant viruses can establish latent infection in neurons, carry multiple foreign DNA sequences and deliver genes into neurons with high efficiency. Unlike amplicons, recombinant viruses do not require a helper virus.

Amplicons: HSV amplicons (Frenkel *et al.,* 1982; Stow & McMonagle, 1982; Spaete & Frenkel, 1982, 1985) are bacterial plasmids each containing a HSV-1 origin of DNA replication and a HSV-1 packaging signal (Vlazny & Frenkel, 1981; Vlazny *et al.,* 1982; Stow *et al.,* 1983). A foreign gene of interest, together with an appropriate promoter and polyadenylation signal, can be inserted into the amplicon. Introduction of such a plasmid with HSV helper virus into cultured cells facilitates replication of amplicon DNA and it's packaging as concatemeric molecules into HSV virions. Cells produce virions containing either the helper virus DNA or the amplicon DNA, which are capable of infecting other cells. Amplicons are technically easier to construct than recombinant viruses because only plasmid DNA is genetically manipulated. Although amplicons can accomodate multiple copies of the gene of interest which can be delivered into cells with high efficiency, they have disadvantages compared with recombinant viruses. First, amplicons contain

only a small amount of HSV genetic information and cannot be grown in the absence of helper virus and therefore cannot be plaque purified. Second, although the amplicon and helper virus genomes are different, their identical virion structure means that they cannot be separated and it is therefore not possible to obtain pure amplicon virus stocks.

1.9.2 Use of HSV vectors to express foreign genes in neurons *in vitro* and *in vivo*

Promoter elements derived from HSV-1, other herpesviruses and cellular genes have been used to regulate expression of foreign genes introduced into neurons by HSV vectors (Desrosiers *et al.,* 1985; Ho & Mocarski, 1988, 1989; Dobson *et al.,* 1989, 1990). The choice of promoter, the position of insertion of the foreign gene of interest and the presence or absence of specific viral genes may all influence the stability of gene expression.

Several groups have shown that amplicons can be used to deliver genes into neurons *in vitro* (Geller & Breakefield, 1988; Geller & Freese, 1990; Geller *et al.,* 1990, 1993, 1995; Chang *et al.,* 1991; Battleman *et al.,* 1993; Bergold *et al.,* 1993; Geschwind *et al.,* 1994) and *in vivo* (Federoff *et al.,* 1992; Ho *et al.,* 1993; During *et al.,* 1994; Kaplitt *et al.,* 1994). Amplicons containing the *LacZ* gene under the control of HSV promoters have been shown to express β-galactosidase in cultured PNS and CNS neurons for up to several weeks after infection (Geller & Breakefield, 1988; Geller & Freese, 1990; During *et al.,*

1994; Kaplitt *et al.,* 1994; Smith *et al.,* 1995). HSV IE gene promoters may drive foreign gene expression more efficiently than L gene (eg. gC) promoters (Smith *et al.,* 1995). Long term detection of β-galactosidase in neurons cannot be explained by persistence of the reporter protein alone (Smith *et al.,* 1995). Hence, it is thought that HSV IE gene promoters in amplicons remain active in neurons for up to several weeks after infection.

Like amplicons, recombinant HSVs have been shown to be suitable for delivery of foreign genes into sensory neurons *in vitro* and *in vivo*. A potential practical application of HSV mediated gene transfer is enzyme replacement therapy in neurons. For instance, transient expression of human phosphoribosyl transferase (HPRT) mRNA in mouse brainstem neurons was achieved by infection with a thymidine kinase deficient (TK^-) HSV vector containing the human HPRT gene under the control of the viral TK promoter (Palella *et al.,* 1988, 1989).

Using recombinant HSVs, several groups have evaluated the effect of different viral or cellular promoters and the genomic location of their insertion on *LacZ* expression. For example, a recombinant virus containing the rat type II sodium channel promoter controlling *LacZ* was shown *in vitro* and *in vivo* to result in β-galactosidase expression in cells of neuronal origin, consistent with the neuron-specific activity of this promoter (Maue *et al.,* 1990). In addition, as with endogenous sodium channel mRNA, *LacZ* expression can also upregulated

with cAMP and nerve growth factor (Mandel *et al.,* 1988). Ho & Mocarski (1988) showed that corneal inoculation of a TK^- HSV vector containing the *LacZ* gene under the control of the HSV-1 ICP4 and TK promoters, resulted in transient expression of β-galactosidase in mouse trigeminal ganglionic neurons. Dobson *et al.* (1990) constructed an ICP4 defective vector with *LacZ* under the control of the Moloney murine leukemia virus long terminal repeat (moMLV-LTR) promoter and demonstrated that a non-HSV promoter could result in stable β-galactosidase expression in sensory neurons following footpad inoculation. Stable β-galactosidase expression was also detected in a small proportion of motor neurons of mice following tongue inoculation. Unlike sensory neurons, motor neurons are not a prominent site of latent HSV infection, which may explain the low number of β-galactosidase expressing motor neurons detected in this study. There have been several studies suggesting that the moMLV-LTR promoter may be more effective than other promoters for expression of foreign genes in neurons *in vivo* (Huang *et al.,* 1992; Andersen *et al.,* 1992; Davar *et al.,* 1994).

Several groups have assessed foreign gene expression driven by HSV-1 LAT promoters in neurons *in vivo*. The rationale for using this promoter was that LATs are abundantly expressed during latency (Stevens *et al.,* 1987). Stable foreign gene expression in neurons has been achieved using LAT promoters. For example, Dobson *et al.* (1989) demonstrated stable β-globin mRNA expression by *in situ* hybridization in sensory neurons infected with a

recombinant HSV containing the β-globin gene inserted downstream of a TATA box element, which is necessary for expression of LATs. Ho and Mocarski (1989) constructed a recombinant HSV in which the *LacZ* gene was inserted downstream of the 5' end of the abundant 2kb major LAT species. Following corneal scarification of mice with this virus, up to one percent of trigeminal ganglionic neurons stably expressed β-galactosidase eight weeks after inoculation.

Several issues related to the use of HSV vectors for delivery of genes into neurons need further investigation. It is not clear whether the location of a foreign gene in the viral genome influences the stability of it's expression, despite early expectations that genes inserted into the LAT locus would be preferentially expressed during latency. It is also unclear what viral or cellular factors influence the regulation of gene expression. Nonetheless, the ability of HSV to selectively target neurons makes it a powerful tool for foreign gene delivery to the nervous system.

1.10 Aim of project

The aim of the project was to determine whether HSV infection interferes with expression of MHC-Ia molecules on the surfaces of primary sensory neurons *in vivo*.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Cells

Vero (African green monkey kidney) cells (ATCC CCL 81), IMR-32 (ATCC CCL 127; derived from a human neuroblastoma) and Neuro-2a cells (ATCC CCL 131; derived from a spontaneous tumour of a strain A albino mouse consisting of neuron-like cells and small round stem cells) were grown in HDMEM supplemented with 10% fetal calf serum (FCS).

EL4 (H-2^b mouse lymphoma) cells and P815 (H-2^d mouse mastocytoma) cells (ATCC TIB 64) were grown in HDMEM supplemented with 10% heat inactivated FCS and were provided by Dr I Kotlarski (Dept. of Microbiology & Immunology, University of Adelaide, South Australia, Australia).

L929 (H-2^k mouse fibroblast) cells were provided by Dr I Kotlarski. NS1 cells, a non-secreting B-myeloma cell line (ATCC CRL 1732) were obtained from the Commonwealth Serum Laboratories, Melbourne, Australia. K562 (human myelogenous leukemia) cells were provided by Dr L Ashman (Hanson Centre for Cancer Research, IMVS, South Australia, Australia). HUT 78 (human T lymphoblastoid) cells were provided by Dr L Peng (HIV Research Lab, IMVS, South Australia, Australia). All cells were grown in RPMI supplemented with

10% FCS.

All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

2.1.2 Antibodies

S19.8 is a murine IgG_{2b} monoclonal antibody against the C57BL allele of β_2m (Tada *et al.,* 1980) and was a gift from Dr J Allison (Walter Eliza Hall Institute, Victoria, Australia).

34-1-2S is a murine IgG_{2a} anti-H-$2K^{d/b}/D^d$ monoclonal antibody derived from the hybridoma ATCC HB79 (ATCC, Rockville, Maryland, USA).

M1/9.3.4.HL.2 is a rat IgG_{2a} monoclonal antibody against the mouse common leukocyte antigen (CD45) derived from the hybridoma ATCC TIB122 and was provided by Dr S Robertson (Dept. of Obstetrics & Gynaecology, University of Adelaide, South Australia, Australia).

ID4.5 is a murine monoclonal antibody against a salmonella antigen (O'Connor & Ashman, 1982) provided by Dr L Ashman (Hanson Centre for Cancer Research, IMVS, South Australia, Australia).

7B6C is a murine IgG_{2a} monoclonal antibody against human β_2m and was provided by Dr L Ashman (Hanson Centre for Cancer Research, IMVS, South Australia, Australia).

YN1/1.7.4 is a rat IgG_{2a} monoclonal antibody against mouse ICAM-1 (Takei, 1985) derived from the hybridoma ATCC CRL 1878 and was provided by Dr N King (Dept. of Pathology, University of Sydney, NSW, Australia).

Anti-TAP-2 rabbit antiserum against the recombinant mouse TAP-2 protein (Suh *et al.,* 1994) was provided by Dr Y Yang (Scripps Research Institute, California, USA).

2.1.3 Viruses

Experiments were done with HSV-1 strains C3b or S-130. C3b is a genetically engineered recombinant HSV which contains the *Escherichia coli LacZ* gene, under the control of the immediate early (IE-1) promoter/enhancer of human cytomegalovirus (HCMV), inserted within the major LAT region of HSV-1 strain SC16. C3b was constructed from strain SC16 and has the same growth characteristics as SC16 in cell culture and in mouse ears, ganglia, brainstems or spinal cords (S Efstathiou, unpublished).

SC16 is a well characterized oral isolate of HSV-1 (Hill *et al.,* 1975). It is neurovirulent after cutaneous inoculation of mice, producing a transient productive infection in skin and sensory ganglia (Harbour *et al.,* 1981; Tullo *et al.,* 1982; Simmons & Nash, 1984; Simmons & LaVista, 1989; Simmons, 1989; Speck & Simmons, 1991, 1992), followed by a stable latent infection in primary sensory neurons that is indistinguishable at the molecular level from that seen in humans (Efstathiou *et al.,* 1986).

S-130 is a genetically engineered recombinant HSV containing a MHC-Ia heavy chain (H-2K^d) cDNA inserted within the major LAT region of HSV-1 strain C3b (A Abendroth, honours dissertation, 1992; see methods 2.2.1 and Figure 2.2).

Virus working stocks were grown and titrated in Vero cells and stored in 0.2ml samples at -70°C until required. Samples were thawed rapidly, diluted as required in HDMEM-MM and kept on ice for up to 30 mins before use.

2.1.4 Mice

Adult female Balb/c (H-2^d), CBA (H-2^k), C3H/HEJ (H-2^k) and C57BL10 (H-2^b) mice were obtained from the Specific Pathogen-Free facility, Animal Resource Centre, Perth, Western Australia, where genetic authenticity checks were carried out 6 monthly. All mice were used at greater than 8 weeks of age.

2.1.5 Plasmids

Plasmids were propagated as described by Maniatis *et al.* (1982).

pBS-4, a gift from Dr S Efstathiou (Cambridge University, U.K.) contains a 1.8kb *Bam*HI-*Sal*I fragment from the IE175 gene of HSV-1 strain KOS cloned into pBluescribe (Stratagene, USA) (Figure 2.1).

pSLAT-1, a gift from Dr S Efstathiou (Cambridge University, U.K.) contains a 4.8kb *Bam*HI-*Pst*I fragment from the LAT region of HSV-1 strain SC16 cloned into pBluescribe (Stratagene, USA).

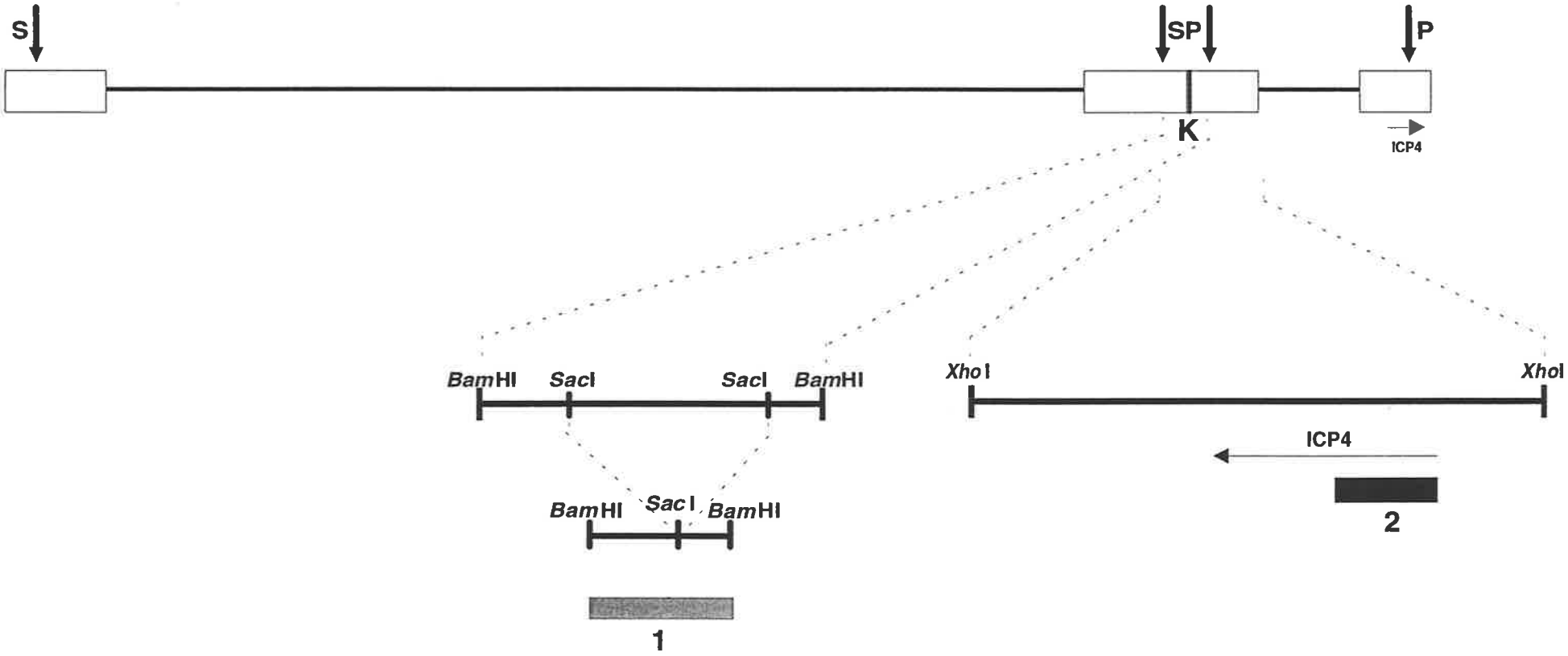
pBKSP1, a gift from Dr A Puga (National Institutes of Health, USA) was made by cloning into pBR322 a 2.4kb DNA fragment constructed from the 5.9kb *Bam*HI-K junction fragment of HSV-1 strain KOS, from which an internal 3.5kb *Sac*I fragment was deleted (Figure 2.1).

EH3KpBST (Horley *et al.,* 1989), a gift from Dr F Takei (University of British Columbia, Canada) contains a 2.1kb mouse ICAM-1 cDNA (K4-1.1), minus it's 3' untranslated region, cloned into pBluescript (Stratagene, USA).

pAL41 (Alonso *et al.,* 1986), a gift from Dr S Alonso (Pasteur Institute, France) contains a 1.15kb *Pst*I fragment of mouse β-actin cDNA, inserted into the *Pst*I site of pBR322.

Figure 2.1

Genome structure of HSV-1 showing the positions of cloned viral fragments used as probes in Southern and northern blot hybridization experiments. Box 1 shows the 2.4kb junction-specific region used to construct pBKSP1, consisting of the *Bam*HI-K fragment (K) from which has been deleted a central 3.5kb *Sac*I fragment. The junction fragment K consists of the fusion of two *Bam*HI terminal fragments P and S. Box 2 shows the position of the 1.8kb *Bam*HI-*Sal*I fragment from the IE175 gene encoding ICP4.


S
SP
P
K
ICP4
BamHI
SacI
SacI
BamHI
BamHI
SacI
BamHI
1
XhoI
XhoI
ICP4
2

mtp-1 and mtp-2 (Deverson *et al.,* 1990), gifts from Dr E Deverson (Cambridge Research Station, UK) contain a 2.7kb rat TAP-1 cDNA and a 2.4kb rat TAP-2 cDNA, respectively, each cloned into the *Eco*RI site of pBluescript KS(+).

pGEM3β_2(A), a gift from Dr R Pereira (IMVS, Adelaide, South Australia) contains a 700bp mouse β_2m cDNA derived from pBRCB4 (Parnes & Seidman, 1982), cloned into the *Pst*I site of pGEM3.

pRC/CMV (In Vitrogen, USA) is a neomycin-resistant eukaryotic expression vector which contains a multiple cloning site flanked by the HCMV IE-1 promoter/enhancer and the bovine growth hormone (BgH) polyadenylation signal sequence.

pLK^d-R contains a H-2K^d expression cassette comprised of the HCMV IE-1 promoter/enhancer, a 1.1kb H-2K^d cDNA and a BgH polyadenylation signal sequence. The H-2K^d expression cassette was cloned into the *Hpa*I site of pSLAT-1, resulting in HSV-1 LAT sequences flanking the H-2K^d expression cassette (A Abendroth, honours dissertation, 1992).

pGEMAA-C was constructed by cloning a 381bp *Bam*HI fragment from pRC/CMV into pGEM-4Z (Promega, USA) using methods described by Maniatis *et al.* (1982). The orientation of cloning was such that transcripts from the T7 promoter are complementary (anti-sense) to RNA transcripts derived from the H-2K^d expression cassette used in the construction of HSV-1 strain S-130.

pBSAA-R was constructed by cloning a 414bp *Sma*I-*Hind*III fragment of pGEMAA-C into pBluescribe. Transcripts from the T7 promoter are the same sense as RNA transcripts derived from the H-2K^d expression cassette used in the construction of HSV-1 strain S-130.

2.2 METHODS

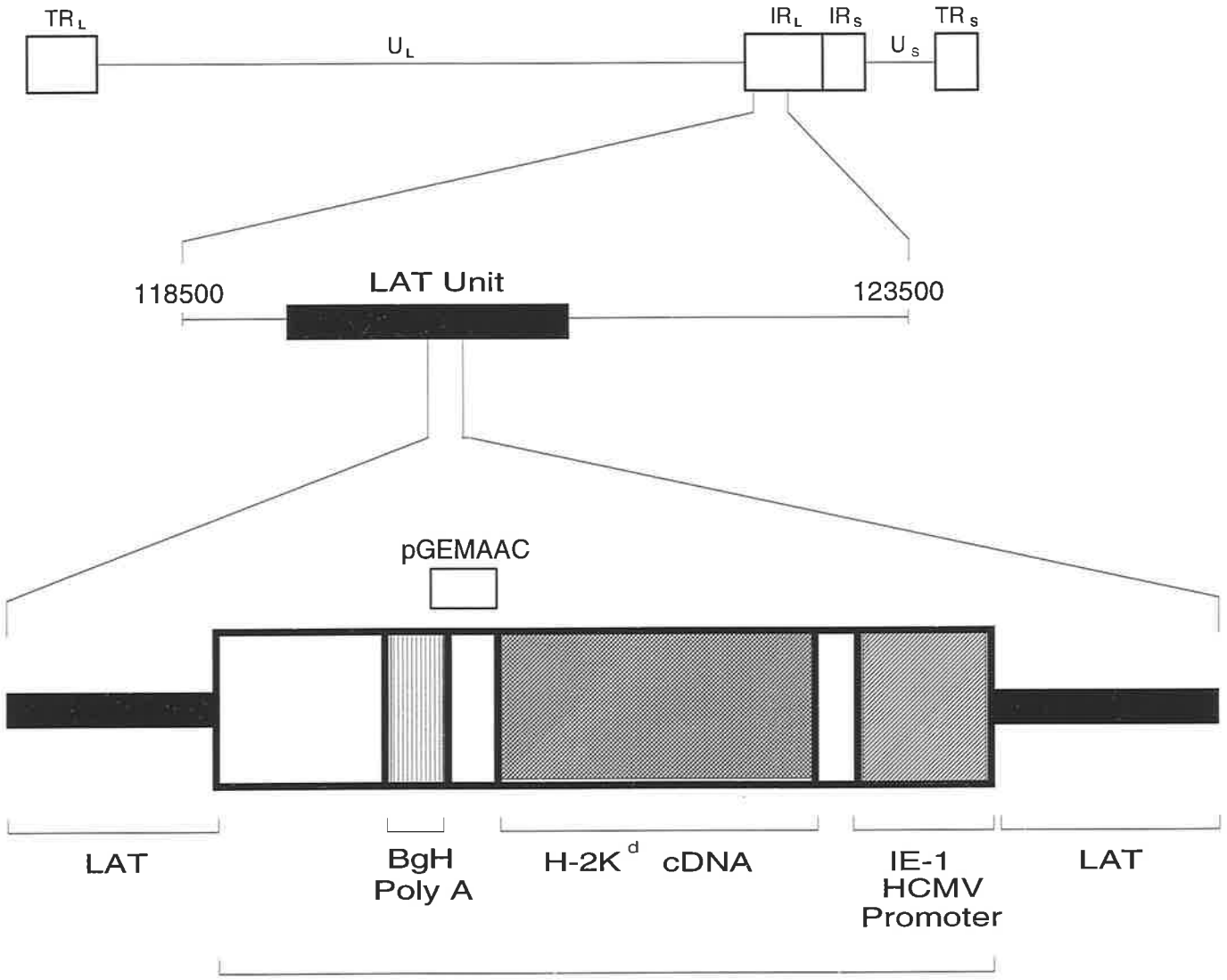
2.2.1 Construction of a recombinant HSV-1, strain S-130

S-130 contains a H-2K^d expression cassette and was constructed as follows. The H-2K^d expression cassette, pLK^d-R, was constructed using a complete, well characterized 1.1kb H-2K^d heavy chain cDNA (Lalanne *et al.,* 1983) placed under the control of a strong constitutive IE-1 HCMV promoter/enhancer and flanked by HSV-1 major LAT sequences (Figure 2.2). pLK^d-R transfected Vero cells were shown by northern blot analysis to synthesize full length H-2K^d RNA transcripts of the predicted size (1.8kb) from the H-2K^d expression cassette (A Abendroth, honours dissertation, 1992).

The H-2K^d expression cassette was inserted into both genomic copies of the LAT unit of the parental HSV-1 strain C3b by homologous recombination between HSV-1 LAT sequences in pLK^d-R (flanking the H-2K^d expression cassette) and viral DNA from HSV-1 strain C3b (Figure 2.2). The LAT locus was chosen as the insertion site because disruption of this region does not inhibit viral replication in cultured cells, mouse peripheral tissues and neurons (Ho & Mocarski, 1989).

Figure 2.2

Structure of the recombinant HSV-1 strain S-130. The HSV-1 genome (top line) contains long and short unique regions (U_L & U_S, respectively) flanked by inverted repeats (TR_L/IR_L & TR_S/IR_S). The region spanning nucleotides 118500-123500 is expanded to show the position of LAT sequences (solid line) and site of insertion of the H-$2K^d$ expression cassette. The H-$2K^d$ expression cassette flanked by HSV-1 LAT sequences (pLK^d-R) was inserted within the major LAT region of the HSV-1 genome. A 381bp subclone (open box labelled pGEMAA-C), specific for transcripts derived from the H-$2K^d$ expression cassette, was used as a probe for northern blot and *in situ* hybridization experiments.


TR L
U L
IR L
IR S
U S
TR S
HSV-1
118500
LAT Unit
123500
pGEMAAC
pLK^{d}-R
LAT
BgH
Poly A
H-2K^{d} cDNA
IE-1
HCMV
Promoter
LAT
EXPRESSION CASSETTE

Transfection procedure: Transfection of Vero cells with pLK^d-R DNA (3μg) and purified C3b virion DNA (10μg) was done on subconfluent monolayers using a modification of the calcium phosphate procedure of Chen & Okayama (1987). Monolayers (in 25cm^2 flasks) were washed three times briefly with hepes buffered saline (HBS). Prior to transfection DNA samples (20μl C3b DNA & 5μl pLK^d-R) were mixed with 0.5ml HBS and 30μl 2M $CaCl_2$ and incubated for 12 mins at room temperature. This DNA solution (~0.5ml) was added dropwise to the cells, swirled gently and incubated for 10 mins at room temperature. Following incubation, cells were overlaid with 4ml of HDMEM-MM and incubated for 4-6 hours at 37°C in a 5% CO_2 atmosphere. The medium was removed and 2ml of 20% dimethyl sulphoxide (DMSO) in HDMEM-MM was added dropwise to cover the cell monolayer and incubated for 2 mins. The DMSO medium was removed and cells immediately rinsed with HDMEM-MM (4x3ml washes). After addition of 5ml HDMEM-GM, cells were incubated at 37°C in a 5% CO_2 atmosphere. Cells were harvested 3 days after transfection and sonicated. The resulting virus suspension was stored in 0.2ml aliquots at -70°C.

Detection of β-galactosidase expression in virally infected cells: The site of homologous recombination was designed to cause disruption of the *LacZ* locus in C3b, therefore recombinant viruses would produce white plaques in the presence of 5-bromo-chloro-3-idolyl-B-D-β-galactopyranoside (X-gal). β-galactosidase staining of viral plaques in cultured cell monolayers was done by

a method similiar to that of Forrester *et al.* (1992). Ten-fold serial dilutions of infectious virus stock were shaken for 1 hour at room temperature with $3x10^6$ Vero cells. Following incubation 3ml of HDMEM-GM containing 1% carboxymethylcellulose (CMC) (Sigma, USA) was mixed thoroughly with each sample. The cell suspension was plated in 60mm tissue culture dishes (Nunc, Denmark) and incubated for 2.5 days at 37°C in a 5% CO_2 atmosphere. Cell monolayers were washed with 0.1M Tris-HCl pH 7.5, then fixed with 0.5% glutaraldehyde in PBS for 15 mins at room temperature. Following fixation, the cells were washed in 2mM $MgCl_2$ in PBS on ice (3x2 min washes) and permeabilized by incubation on ice with a detergent solution (2mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02% Nonidet P-40). After 10 mins the detergent solution was replaced with detergent solution containing 5mM potassium ferricyanide-ferrocyanide and 1mg/ml X-gal (Boehringer Mannheim, Germany). Plates were incubated for 3-4 hours at 37°C, washed twice in PBS, counterstained with 1% neutral red, and blue and white plaques were counted using a low-power dissecting microscope.

Plaque purification: Following co-transfection of Vero cells with plasmid and viral DNA, non-β-galactosidase producing recombinant virus was selected by plaque purification. Vero cells were infected with transfection progeny virus at an appropriate dilution to result in approximately 20 plaques per 60mm tissue culture dish (Nunc, Denmark). After 2 days, white plaques were detected using an overlay of low melting temperature ME-agarose containing 300μg/ml X-gal

(Boehringer Mannheim, Germany). Agar plates containing white plaques were picked with a pasteur pipette and incubated at 4°C for at least 1 hour in HDMEM-MM. A further three rounds of plating and plaque picking were done in order to obtain pure white stocks.

Characterization of recombinant virus (S-130) by Southern blot hybridization: Confluent Vero cell monolayers were infected at 5 plaque forming units (pfu)/cell with C3b or putative recombinant viruses and cells were harvested 24 hours after infection. DNA was extracted from cells by the method described in section 2.2.19. The genome structure of recombinant viruses was tested by Southern blot hybridization of *Eco*RI digested DNA using a ^{32}P-labelled probe generated from the H-2K^d cDNA. S-130 was shown to contain the H-2K^d expression cassette in both genomic copies of the LAT unit of parent virus C3b (A Abendroth, honours dissertation, 1992).

2.2.2 Titration of infectious virus

Plaque assays were done in duplicate by the suspension method of Russell (1962). Serial ten-fold dilutions of tissue homogenates or virus samples in 2ml of HDMEM-MM were shaken for 1 hour at room temperature with $3x10^6$ Vero cells. Following incubation, 3ml of HDMEM-GM containing 1% CMC (Sigma, USA) was mixed thoroughly with each sample. Samples were plated in 60mm tissue culture dishes (Nunc, Denmark) and incubated at 37°C for 2.5 days in 5% CO_2 atmosphere. Cell monolayers were fixed with 10% formal

saline for 15 mins, stained with 0.1% toluidine blue for 10 mins and washed briefly with tap water. Plaques were counted using a low-power dissecting microscope.

2.2.3 Inoculation of mice

Prior to infection, the left flanks of mice were shaved and depiliated with Nair (Carter Wallace, Australia). A 10μl drop of virus suspension containing 1.0×10^5 pfu was applied to the posterior flank (over the spleen tip) corresponding to the 8th thoracic dermatome (T8). Skin was scarified 20 times through the virus suspension using a 27 gauge needle (Terumo, Japan), giving a total scarified area of approximately $4mm^2$ (Simmons & Nash, 1984; Simmons, 1985; Simmons & LaVista, 1989; Speck & Simmons, 1991). Infected mice developed characteristic band-like zosteriform lesions 5-6 days after virus inoculation. For ear inoculation, 20μl of virus suspension containing 1.0×10^5 pfu was injected subcutaneously into the pinna of the left ear of anaesthetized mice (Hill *et al.*, 1975).

2.2.4 Removal of infected tissue from mice

Mice were killed by CO_2 asphyxiation. The viscera were removed through an anterior midline incision, exposing the anterior surface of the spinal column. The 13th thoracic vertebrae (T13) were identified by their articulation with the lowest ribs. Other vertebrae were identified according to their position relative to T13. Fine forceps were used to tease apart the vertebrae at the intervertebral

discs in order to expose and remove consecutive dorsal root ganglia (T6-T11) ipsilateral to the inoculation site. Ganglia were pooled and placed immediately into PBS on ice. In some experiments skin samples encompassing the inoculation site were also taken.

2.2.5 Preparation of cell suspensions from thoracic dorsal root ganglia

Pooled ganglia were washed 3 times in PBS and cells were enzymatically dissociated with collagenase/dispase (10mg/ml) (Boehringer Mannheim, Germany) for 1.5 hours at 37°C with occasional gentle pipetting. For rosetting experiments, ganglionic cells were washed in PBS and resuspended in HDMEM containing 0.5% bovine serum albumin (BSA). Cells to be used for immunohistochemical staining were resuspended in PBS and smeared onto glutaraldehyde activated aminopropyltriethoxysilane (APES) coated slides. Cell smears were dried overnight at room temperature prior to fixation.

2.2.6 Preparation of paraffin sections

Dorsal root ganglia were fixed for 2 hours at room temperature in freshly prepared periodate-lysine paraformaldehyde (PLP) (McLean & Nakane, 1974), washed twice in 50% ethanol, rolled together into a ball and placed into tissue specimen cassettes. To minimize shrinkage of primary sensory neurons, ganglia were dehydrated over several hours using graded ethanol solutions. Ganglia were paraffin embedded using a Shandon processing machine in the Division of Tissue Pathology, IMVS, Adelaide.

Prior to use, glass microscope slides and coverslips were washed with chromic acid (10% sulphuric acid/0.35M potassium dichromate) for 24 hours, then rinsed 5 times with double distilled water (DDW) and allowed to dry. Slides were dipped in 2% APES in ethanol for 5-10 seconds, rinsed 3 times in ethanol, 3 times in DDW, dried in air and stored for up to 3 months before use. Immediately before use slides were immersed in 10% glutaraldehyde in PBS for 30 mins to activate APES, rinsed twice in DDW and dried in air at room temperature. Glutaraldehyde activated APES was used to maximize adherance of tissues and cells to slides (Maples, 1985). Ganglionic sections (5μm) were cut from paraffin embedded blocks, collected onto glutaraldehyde activated APES coated slides, dried overnight at 37°C and stored until required.

2.2.7 Preparation of frozen tissues

Dorsal root ganglia were embedded in optimum cutting temperature (OCT) compound (Miles Inc., Iowa, USA) and frozen in liquid nitrogen-cooled isopentane (Menco, 1986). Frozen tissues were stored at -70°C for up to 7 days. Sections (6μm) were cut at -20°C using an Ames cryostat II (Miles Inc., Iowa, USA), collected onto glutaraldehyde activated APES coated slides, dried in air overnight, fixed at 4°C in acetone for 10 mins and finally dried for 1 hour at room temperature. Sections were stored for up to 3 days after fixation.

2.2.8 *In vitro* reactivation of latent virus from thoracic dorsal root ganglia

HSV was reactivated from latently infected thoracic dorsal root ganglia using well established explant culture techniques (Stevens & Cook, 1971). Briefly, T6-T11 dorsal root ganglia were incubated in 1ml of HDMEM-MM for 5 days at 37°C in a 5% CO_2 atmosphere, homogenized and tested on Vero cells for the presence of infectious virus (see section 2.2.2).

2.2.9 One-step virus growth curves

Vero cell monolayers in 60mm tissue culture dishes (Nunc, Denmark) were infected at a multiplicity of 5 pfu/cell in 1ml of HDMEM-MM. After a 1 hour adsorption period, the cells were washed with HDMEM-MM and incubated at 37°C with HDMEM-MM. At various times after infection cells were harvested by scraping, lysed by repeated freezing and thawing and tested on Vero cells for infectious virus (see section 2.2.2).

2.2.10 Infectious centre assay (ICA)

Vero, IMR-32 and K562 cells were infected at 10 pfu/cell. After 1 hour of virus adsorption the inoculum was replaced with HDMEM-MM or RPMI-MM. Cells were washed twice in 0.25% trypsin, twice in HDMEM-GM and resuspended in HDMEM-MM ($2x10^6$ cells/ml). Serial ten-fold dilutions of infected cells were done in HDMEM-MM and mixed thoroughly with 3ml of HDMEM-MM containing 1% CMC (Sigma, USA). Samples were poured onto preformed Vero cell monolayers ($2x10^6$ cells/dish) in 60mm tissue culture

dishes (Nunc, Denmark) and incubated at 37°C for 3 days in 5% CO_2 atmosphere. Cell monolayers were fixed with 10% formal saline for 15 mins, stained with 10% toluidine blue for 10 mins and washed briefly with tap water. Plaques were counted using a low-power dissecting microscope.

2.2.11 Preparation of HSV infected cells for immunohistochemistry and rosetting

Vero, L929, K562 and IMR-32 cells grown in 25cm^2 flasks (Corning Inc., New York) were infected at 10 pfu/cell. After 1 hour of virus adsorption the inoculum was replaced with HDMEM-GM or RPMI-GM. Cells were harvested 5 hours after infection, washed and resuspended in PBS ($4x10^6$ cells/ml) and smeared onto glutaraldehyde activated APES coated slides. Smears were dried overnight at room temperature and fixed in methanol at 4°C for 15 min. For rosetting experiments, harvested cells were washed and resuspended in HDMEM containing 0.5% BSA ($4x10^6$ cells/ml).

2.2.12 Immunohistochemical detection of HSV antigens

HSV antigens were detected in tissue sections and cell smears using a peroxidase-anti-peroxidase (PAP) method (Moriarty *et al.*, 1973; Sternberger, 1979; Boenisch, 1980). All antisera were purchased from Dakopatts, Denmark. The primary antibody was rabbit anti-HSV infected cells, diluted 1:50. Binding of primary antiserum was detected using swine antiserum against rabbit immunoglobulin (Ig), diluted 1:25, followed by rabbit PAP complex, diluted

1:200. All antisera were diluted in Tris buffered saline (TBS) containing 10% normal swine serum (NSS) and all reactions were allowed to proceed for 30 mins in a humidified atmosphere at 37°C, with a 10 min wash in TBS between each step. Peroxidase activity was detected by immersing slides for 4 mins in the dark in a chromogen solution containing 0.5mg/ml 3,3'-diaminobenzidine (DAB) and 0.1% H_2O_2. The reaction was stopped by thorough rinsing with tap water. Sections were lightly counterstained with rapid haematoxylin, dehydrated using graded alcohol solutions and finally washed in xylene. Coverslips were attached with DePex (BDH chemicals, Australia).

2.2.13 Immunohistochemical detection of H-2K^d antigens

H-2K^d antigens were detected in cell smears with a PAP method using 34-1-2S as the primary antibody. The optimum dilution of this antibody was determined by titration on a H-2K^d expressing cell line, P815. Binding of primary antibody was detected using goat-anti-mouse Ig, diluted 1:50 (Dakopatts, Denmark), followed by mouse PAP complex, diluted 1:100 (Dakopatts, Denmark). All antisera were diluted in TBS containing 10% normal goat serum (NGS) and all reactions were allowed to proceed for 30 mins in a humidified atmosphere at 37°C, with a 10 min wash in TBS between each step. Peroxidase activity was detected as described in 2.2.12.

2.2.14 Immunohistochemical detection of TAP-2 antigens

TAP-2 antigens were detected on frozen ganglionic sections and cell smears using a PAP method. The dilution of the primary antibody, anti-TAP-2 rabbit antiserum, was determined by titration on a TAP-2 expressing cell line, P815. Binding of primary antibody was detected using swine-anti-rabbit Ig, diluted 1:25 (Dakopatts, Denmark), followed by rabbit PAP complex, diluted 1:200 (Dakopatts, Denmark). All antisera were diluted in TBS containing 20% NSS and all reactions were allowed to proceed for 30 mins in a humidified atmosphere at 37°C, with a 10 min wash in TBS between each step. Peroxidase activity was detected as in 2.2.12.

2.2.15 Rosetting assays

Direct rosetting: Antibodies 34-1-2S or ID4.5 were coupled to sheep red blood cells (SRBC) as described previously (Parish & McKenzie, 1978; Goding, 1976 a) with the following modifications: monoclonal antibodies were used at 400μg/ml and chromic chloride at 0.03125% instead of 0.1%. Rosetting was done by incubating 25μl of antibody coupled SRBC with 10^5 target cells in HDMEM containing 0.5% BSA in u-well microtitre plates (Flow Laboratories, USA) for 30 mins. The cell suspensions were mixed gently and sedimented at 200 x g for 1min at 4°C to encourage rosette formation. After 30 mins on ice, cells were gently resuspended and mixed with 50μl of 0.1% methyl violet. Samples were examined in cell counting chambers (Hycor Biochemical Inc., USA) for rosette forming cells (RFCs).

Indirect rosetting: Protein A was coupled to SRBC (prot A-SRBC) as described previously (Sandrin *et al.*, 1978). Rosetting was done by incubating 50μl of target cells with 50μl of primary antibody (34-1-2S, S19.8, 7B6C or ID4.5) in u-well microtitre plates (Flow Laboratories, USA) for 10 mins. Cells were washed twice in HDMEM containing 0.5% BSA and resuspended in 50μl of HDMEM containing 0.5% BSA. 50μl prot A-SRBC were added and incubated at room temperature for 10 mins. The cell suspensions were mixed gently and sedimented at 200 x g for 1 min at 4°C to encourage rosette formation. After 10 mins cells were gently resuspended and mixed with 50μl of 0.1% methyl violet. Samples were examined in cell counting chambers (Hycor Biochemical Inc., USA) for RFCs.

An additional step was necessary when rat IgG_{2a} antibodies, which do not bind to protein A, were used. Target cells were incubated with 50μl of undiluted hybridoma culture supernatants (from YN1/1.7.4. or M1/9.3.4.HL.2) in u-well microtitre plates (Flow Laboratories, USA) for 10 mins. Cells were washed twice in HDMEM containing 0.5% BSA, resuspended in 50μl goat anti-rat Ig (diluted 1:25 in HDMEM containing 0.5% BSA) (Dakopatts, Denmark) and incubated at room temperature for 20 mins. Cells were washed twice and resuspended in 50μl HDMEM containing 0.5% BSA, mixed with 50μl prot A-SRBC and tested for RFCs as described above.

2.2.16 Preparation of spleen cell suspensions and capping of surface immunoglobulin on lymphocytes

Spleens from Balb/c mice were pushed through a stainless steel sieve and cells collected in 5ml of RPMI-GM. Red blood cells were removed from the cell suspension by ficoll density gradient centrifugation as follows: 5ml of spleen cell suspension was layered onto 5ml of Lymphoprep (Nycomed, Pharma AS, Norway) in a 10ml centrifuge tube (Corning Inc., New York). The gradient was centrifuged at room temperature for 20 mins at 800 x g. Interface cells (lymphocytes) were removed with a glass pipette, mixed with 10ml of RPMI-GM, pelleted by centrifugation at 200 x g for 10 mins and washed twice in RPMI-GM. The resulting mouse splenic lymphocytes were counted and resuspended to a final concentration of $1x10^7$ cells/ml in RPMI-GM.

Capping of surface Ig on mouse spleen lymphocytes was done as follows: $5x10^6$ cells (in 0.5ml RPMI-GM) were mixed with goat anti-mouse Ig (50μg/ml; Dakopatts, Denmark) and incubated for 75 mins at 37°C in a humidified atmosphere of 5% CO_2 in air. The capping reaction was interrupted by adding nine volumes of cold RPMI-GM and cells were pelleted at 300 x g for 5 mins. Spleen cells were washed 3 times in RPMI-GM at 4°C and finally resuspended for rosetting to a concentration of $5x10^6$ cells/ml in RPMI containing 0.5% BSA.

2.2.17 Mixed lymphocyte culture (MLC) and ^{51}Cr release assay.

Primary one-way MLCs were set up in $25cm^2$ tissue culture flasks (Corning Inc., New York). $1x10^6$ stimulator cells (P815 or L929 cells) were irradiated with 2000 rads and mixed with $1x10^7$ C57BL10 mouse spleen responder cells ($H\text{-}2^b$) in 5ml of RPMI-GM-2ME containing 5 units of human recombinant interlukin-2 (IL-2) (Boehringer Mannheim, Germany). Flasks were incubated upright for 5 days at 37°C in a humidified atmosphere of 5% CO_2 in air. The resulting effector cells were pelleted by centrifugation (400 x g), resuspended in 0.5ml RPMI-GM and the generation of CTLs assessed using a ^{51}Cr release assay.

Target cells for the ^{51}Cr release assay were treated as follows: L929 and Vero cells ($2x10^6$ cells/0.5ml RPMI-MM) were infected with HSV-1 strain S-130 or C3b at 10 pfu/cell for 2 hours at 37°C in a humidified atmosphere of 5% CO_2 in air and simultaneously labelled with 200μCi of ^{51}Cr. Uninfected P815, L929 and Vero cells were also labelled in the same way. The labelling reaction was interrupted by the addition of nine volumes of RPMI-GM before cells were pelleted at 400 x g for 5 mins, washed three times in RPMI-GM and resuspended to $2x10^5$ cells/ml. Target cells ($2x10^4$ cells/microtitre plate well) were mixed with 100μl of effector cells (generated from MLC), diluted in RPMI-GM to give effector:target cell ratios of 40:1, 20:1, 10:1 and 5:1, in u-well microtitre plates (Flow Laboratories, USA). Triplicate samples were incubated for 5 hours at 37°C in a humidified atmosphere of 5% CO_2 in air.

Spontaneous release of ^{51}Cr was determined by mixing $2x10^4$ target cells with 100μl of RPMI-GM. Total release of ^{51}Cr was determined by mixing $2x10^4$ target cells with 100μl of 1% Triton X-100. Plates were centrifuged at 200 x g for 1 min before 100μl of supernatant from each well was removed and ^{51}Cr release measured on a γ-radiation counter. Cytotoxicity was expressed as mean percent specific activity of ^{51}Cr release and was calculated using the following equation:

% specific activity= (test release-spontaneous release)/(total release-spontaneous release) x100 ± standard error of the mean (SEM) from triplicate assays.

2.2.18 Preparation of lymph node cell suspensions and adoptive transfer into mice

This procedure was done using a modification of the method described by Nash *et al.* (1980 a). Seven days after ear inoculation of Balb/c or CBA mice with 1.0 x 10^5 pfu HSV-1 strain SC16, draining lymph nodes (DLN) ipsilateral to the inoculation site were aseptically removed and gently homogenized. The cell suspension was filtered through a metal gauze mesh and washed twice in HDMEM-MM. CBA mice were sublethally irradiated (600 rads) 24 hours prior to intravenous injection of 2 x 10^7 DLN cells (in 0.2ml HDMEM-MM). Approximately 60 mins after adoptive transfer 1.0 x 10^5 pfu of virus suspension was inoculated into the pinna of the left ear. Control groups

received no cells but were inoculated with virus. At various days after infection, left ears were removed, homogenized and tested on Vero cells for infectious virus (see section 2.2.2).

2.2.19 Extraction of ganglionic DNA

Ganglia were washed twice in PBS and homogenized gently in 300μl TE (10:1) pH 8.0. SDS was added to a final concentration of 0.5% and the lysate was treated with 100μg/ml proteinase K (Boehringer Mannheim, Germany) for two hours at 37°C. Proteins were removed by three phenol-chloroform (1:1) extractions followed by three chloroform extractions. DNA was spooled from the aqueous interface after addition of 1/10 volume of 3M sodium acetate (pH 5.5) and 2.5 volumes of ethanol. Spooled DNA was dried in air and dissolved at 4°C in Commonwealth Serum Laboratories (CSL) water. The quantity and purity of DNA was determined by spectrophotometry. Samples were stored at -20°C until required.

2.2.20 Extraction of ganglionic RNA

To minimize RNA degradation, glassware and materials were acid washed and unless otherwise stated, the extraction procedure was done at 4°C.

Thoracic dorsal root ganglia were frozen rapidly in eppendorf tubes floating on liquid nitrogen. Cultured cells were scraped from tissue culture flasks and washed twice in PBS. Ganglia or cultured cells were homogenized vigorously

in 400μl guanidine acetate buffer (GAB) in a 1ml glass tissue homogenizer. The homogenate was mixed with half it's own volume of ethanol and incubated overnight at -20°C. Samples were microfuged at 12000 x g for 10 mins and the pellet resuspended in 300μl urea-SDS buffer. Proteins were removed by three phenol-chloroform (1:1) extractions followed by three chloroform extractions. RNA was precipitated over 16 hours at -20°C by addition of 1/10 volume of 3M sodium acetate (pH 5.5) and 3 volumes of ethanol. The precipitate was pelleted at 12000 x g for 10 mins and resuspended in CSL water. Samples were reprecipitated overnight at -20°C and the RNA pellet was washed briefly in 70% ethanol, dried and resuspended in CSL water (50μl). The quantity and purity of RNA was assessed by spectrophotometry. Samples were stored at -70°C until required.

2.2.21 Preparation of probes for Southern and northern blot hybridization

Probe sequences were excised from their host plasmids by using the following restriction endonucleases: pBKSP1, *Bam*HI; pBS-4, *Bam*HI-*Sal*I; pAL41, *Pst*I; mtp1, *Hind*III-*Spe*I; mtp2, *Eco*RI; EH3KpBST, *Eco*RI-*Bam*HI; pGem3β_2(A), *Kpn*I-*Hind*III; pGEMAA-C, *Bam*HI. DNA restriction fragments were separated by electrophoresis in 1.0% TAE agarose and the required fragments were purified using Geneclean II (Bio 101 Inc., California, USA). Purified restriction fragments were quantified and checked for correct size by gel electrophoresis using the migration and intensity of ethidium bromide stained

SPP1 *Eco*RI fragments (Bresatec, Australia) for reference. Fragments were labelled with ^{32}P using an *in vitro* random primed DNA labelling procedure (Feinberg & Vogelstein, 1983).

Reaction mixtures contained:

25 ng denatured DNA template

25 μM dATP (Boehringer Mannheim, Germany)

25 μM dGTP (Boehringer Mannheim, Germany)

25 μM dTTP (Boehringer Mannheim, Germany)

50 μCi ^{32}P-dCTP, 3000 Ci/mM (Bresatec, Australia)

1x random hexanucleotide primer reaction mixture (Boehringer Mannheim, Germany)

2 units Klenow enzyme, labelling grade (Boehringer Mannheim)

CSL water to a final volume of 20 μl

After 1 hour at 37°C the reaction was stopped with 1μl 500mM EDTA, pH 8.0. Efficiency of labelling was determined by measuring the incorporation of radioactive label by differential precipitation of DNA using trichloroacetic acid (TCA) (Sambrook *et al.,* 1989). Typically 75-95% of available radiolabel was incorporated during the labelling reaction, resulting in probes with a specific activity of at least 10^9 dpm/μg. Labelled DNA was precipitated by adding 20μl 3M sodium acetate (pH 5.5), 20μl 10 mg/ml sheared salmon sperm DNA, 40μl CSL water and 450μl ethanol. The mixture was stored at -70°C for 4 hours before centrifugation at 12000 x g for 20 minutes. To remove unincorporated

nucleotides the pellet was washed 5 times in ethanol, dried briefly under vaccum and redissolved in 300 μl CSL water. Probes were utilized in hybridization reactions immediately after preparation.

2.2.22 Detection of HSV DNA fragments by Southern blot hybridization

Endonuclease digestion, electrophoresis and Southern blot transfer: Ganglionic DNA samples (10μg) were digested with 40 units of *Bam*HI restriction endonuclease (Amersham, UK) for 2 hours at 37°C. All experiments included DNA extracted from uninfected mice and SPP1 *Eco*RI size markers (Bresatec, Australia) were included to assist in determination of DNA fragment size. A 1/10 volume of DNA tracking dye was added and restriction fragments were separated by electrophoresis (for 16 hours at 2V/cm) in 0.8% agarose in TAE containing 1μg/ml ethidium bromide. To remove excess ethidium bromide the gel was washed in DDW for 10 mins. DNA fragments were visualized and photographed under ultraviolet light (302 nm) (UVP transilluminator, San Gabriel, USA). Ultraviolet light (302nm for 2 mins) was also used to partially nick double stranded DNA. The gel was then shaken gently in denaturation solution for 30 mins and then for 30 mins in neutralization solution, before DNA was transferred to nitrocellulose filters (Schleicher & Schuell Co, Germany) by the technique of Southern (1975). After transfer, filters were rinsed briefly in 2 x SSC, baked under vacuum at 80°C for 2 hours and stored between sheets of Whatman 3MM chromatography paper (Whatman

International Ltd, Maidstone, UK) until required.

Prehybridization and hybridization: Filters were wetted with 6 x SSC, placed into siliconized (Coatasil, Ajax Chemicals, Australia) glass hybridization tubes (Robbins Scientific, USA) and prehybridized at 65°C for 4 hours in 20ml prehybridization solution (for Southern blot hybridization) using a specialized hybridization oven (Robbins Scientific, model 310, USA). Hybridization of probe was done at 65°C (T_m-25°C) for 16 hours in 20ml hybridization solution (for Southern blot hybridization) containing 5 x 10^7 cpm ^{32}P-labelled denatured DNA probe, corresponding to approximately 4ng labelled DNA per ml hybridization solution.

Washing and detection of bound probe: To remove unbound probe, filters were washed 4 times for 1 min and once for 30 mins in 2 x SSC, 0.1% SDS at 65°C before being washed twice at high stringency for 45 mins in 0.1 x SSC, 0.1% SDS at 65°C (T_m-14°C). Filters were wrapped without drying in plastic film (Gladwrap, Glad, Australia) and exposed to storage phosphor screens for 1-2 days. Phosphor screens were processed using a 400 series Phosphor Imager (Molecular Dynamics, California, USA). Bands were visualized and quantified in relation to reconstructions using ImageQuant software (version 3.0, Molecular Dynamics). Reconstructions were made by diluting known amounts of virion DNA with uninfected mouse DNA, to give the equivalent of 5, 0.5 or 0.05 viral genome copies per mouse cell.

2.2.23 Northern blot hybridization

Electrophoresis and northern blot transfer: RNA samples (5μg) from either ganglia or cultured cells were mixed with three volumes of RNA denaturation buffer, heated to 70°C for 5 mins and a 1/10 volume of RNA tracking dye added. Duplicates of each sample were loaded into a 1% agarose gel containing 1 x MOPS buffer and 2% freshly filtered formaldehyde solution, and electrophoresed in 1 x MOPS buffer at 5V/cm for 4 hours. Half the gel containing one complete set of samples was stained with ethidium bromide and photographed, the other half was soaked in 20 x SSC for 1 hour and RNA was transferred to nitrocellulose (Schleicher & Schuell Co, Germany) using a standard northern blotting protocol (Sambrook *et al.*, 1989). After transfer, filters were baked under vacuum for 2 hours at 80°C, rinsed briefly in 2 x SSC to remove excess salt crystals, dried in air at room temperature and stored between sheets of Whatman 3MM chromatography paper (Whatman International Ltd, Maidstone, UK) until required.

Prehybridization and hybridization: To prehybridize filters they were wetted with 5 x SSC and rolled for 4 hours at 42°C in siliconized (Coatasil, Ajax Chemicals, Australia) hybridization tubes (Robbins Scientific, USA) containing 20ml prehybridization solution (for northern blot hybridization). Filters were hybridized for 16-18 hours at 42°C in 20ml hybridization solution (for RNA hybridization) containing 5 x 10^7 cpm ^{32}P-labelled denatured DNA probe, which corresponded to approximately 4ng labelled DNA per ml hybridization

solution.

Washing and detection of bound probe: To remove unbound probe, filters were washed 4 times for 1 min and once for 30 mins in 2 x SSC, 0.1% SDS at 70°C. They were then washed twice at high stringency for 45 mins in 0.1 x SSC, 0.1% SDS at 70°C (T_m-10°C to -12°C). Filters were wrapped without drying in plastic film (Gladwrap, Glad, Australia) and exposed either to X-ray film (X-AR film, Kodak, New York) at -70°C or storage phosphor screens at room temperature for 1-2 days. X-ray film was developed using an Ilfospeed 2240 X-ray processor (Ilford, Australia). Phosphor screens were processed using a 400 series Phosphor Imager (Molecular Dynamics, California, USA) and the relative amounts of RNA in bands was determined using ImageQuant software (version 3.0, Molecular Dynamics).

Stripping of bound probe from nitrocellulose filters: To facilitate reprobing, filters were stripped of bound probe by washing twice in a solution containing 0.05 x SSC, 10mM EDTA pH 7.5 and 0.1% SDS (w/v) for 15 mins at 95°C. Filters were rinsed briefly in 0.01 x SSC at room temperature. Stripping was confirmed by exposing filters to storage phosphor screens prior to reprobing.

2.2.24 *In situ* hybridization (ISH)

Preparation of probes: DIG-labelled riboprobes were used for all ISH reactions. pGEMAA-C or pBSAA-R were linearized by restriction endonuclease digestion such that the cloned fragments encoding probe sequences were downstream of the T7 promoter. Linearization was confirmed by electrophoresis in 1% TAE agarose. Transcription was done using a modification of the procedure described by Promega. Reaction mixtures contained:

1 x transcription buffer (Promega, USA)

5 mM dithiothreitol (DTT) (Promega, USA)

20 units RNAsin (ribonuclease inhibitor, Promega, USA)

0.5 mM rATP (Promega, USA)

0.5 mM rCTP (Promega, USA)

0.5 mM rGTP (Promega,USA)

0.085 μM ^{32}P-rUTP (Bresatec, Australia)

250 μM digoxigenin-11-rUTP (DIG-UTP; Boehringer Mannheim,Germany)

1 μg linearized template plasmid DNA

15 units T3 or T7 RNA polymerase (Promega, USA)

CSL water to give a final reaction volume of 20 μl

Templates were transcribed for 1 hour at 37°C after which they were destroyed by addition of 1 unit of RNAse-free DNAse (Promega, USA) at 37°C for 15 mins. Enzymes were inactivated by chelation of Mg^{++} by addition of 1μl 500mM EDTA, pH 8.0. Labelling efficiency was determined by measuring the incorporation of the radioactive tracer by TCA precipitation (Sambrook *et al.*, 1989). RNA transcripts were precipitated by adding 20μl 4M LiCl, 20μl 10mg/ml sheared salmon sperm DNA, 40μl CSL water and 250μl ethanol. The mixture was stored at -20°C overnight before centrifugation at 12000 x g for 15 mins. The pellet was washed 4 times in ethanol, once in 70% ethanol, dried under vacuum and redissolved in 100μl 10mM Tris-HCl, pH 8.0 containing 1mM DTT and 100 units RNAsin. Probes were stored at -20°C until required.

Preparation of tissue sections: The method used was a modification of the protocol described by Gowans *et al.* (1989). Paraffin sections (5μm) were dewaxed in two changes of xylene for 40 mins, rehydrated gradually through graded ethanol/water mixtures and washed briefly in PBS. Sections were fixed in 0.1% glutaraldehyde in PBS for 30 mins at 4°C, then washed twice for 5 mins in PBS. To improve access of probe to target sequences in tissue sections, proteins were digested with 100μg/ml proteinase K (Boehringer Mannheim, Germany) in 20mM Tris-HCl pH 7.5, 2mM $CaCl_2$ at 37°C for 15 mins (Angerer *et al.*, 1987; Gowans *et al.*, 1989). Sections were washed twice for 5 mins in PBS, refixed in 0.1% glutaraldehyde for 15 mins at 4°C and treated with 0.25% acetic anhydride, 100mM triethanolamine, pH 8.0 for 10

mins at room temperature (Hayashi *et al.,* 1978). Sections were washed twice for 5 mins in PBS, dehydrated gradually in graded ethanol/water mixtures and dried in air before application of hybridization solution.

Nuclease digestions: Where indicated, tissues were treated for 1 hour at 37°C either with 1 unit/μl DNAse 1 (Promega, USA) in 6mM $MgCl_2$ and 40mM Tris-HCl (pH 7.5), or 500μg/ml RNAse A (Pharmacia, Australia) in 2 x SSC. Nuclease digestions were done after proteinase K treatment and tissue sections were thoroughly washed in PBS (5x5 mins) before and after enzyme treatment.

Hybridization: Hybridization solution (20μl) was applied to sections and covered with a 22mm x 22mm siliconized coverslip, taking care not to trap air bubbles. Coverslips were sealed with rubber cement (Super Vulkarn, Maruni Industries, Japan) and slides were incubated at 60°C (T_m-25°C for RNA/RNA hybrids) (Cox *et al.,* 1984; Gowans *et al.,* 1989) for 16 hours. All experiments included sections of uninfected ganglia.

Washing and detection of bound probe: To remove unbound probe, slides were washed with gentle agitation in 2 litres of 2 x SSC for 1 hour and 2 litres of 0.1 x SSC for 1 hour before a stringent wash at 60°C (T_m-12°C for RNA/RNA hybrids) for 30 mins in a solution containing 30% deionized formamide, 0.1 x SSC, 10mM Tris-HCl, pH 7.5. Slides were washed at room temperature in 0.1 x SSC and 10mM Tris-HCl (pH 7.5) for 15 mins before bound DIG-labelled

probe was detected by colourimetric reaction as follows: Slides were washed with gentle agitation for 5 mins in DIG buffer 1 and 30 mins in DIG buffer 2 at room temperature. Anti-DIG-alkaline phosphatase (0.75 units/μl) was diluted 1:750 in DIG buffer 2 before being applied to individual tissue sections and incubated in a prewarmed humidified atmosphere at 37°C for 30 mins. Slides were washed in DIG buffer 1 (4 x 5 mins), then DIG buffer 3 (1 x 5 mins) and finally immersed in freshly prepared colour substrate solution containing 135μl NBT solution and 105μl X-phosphate solution in 30ml DIG buffer 3. Slides were examined for colour development at regular intervals for up to 2 hours, after which the reaction was stopped by washing slides in DDW.

3. CHARACTERIZATION IN CULTURED CELLS OF HSV-1 STRAIN S-130, A RECOMBINANT VIRUS ENCODING A MHC-Ia GENE

HSV-1 strain S-130 contains a H-2K^d expression cassette, comprising a murine MHC-Ia αC (H-2K^d) cDNA under the control of the IE-1 HCMV promoter/enhancer (Abendroth, honours dissertation, 1992; see section 2.2.1). The experiments presented in this chapter describe the characterization of HSV-1 strain S-130 *in vitro*, with particular reference to its growth kinetics and ability to express H-2K^d molecules.

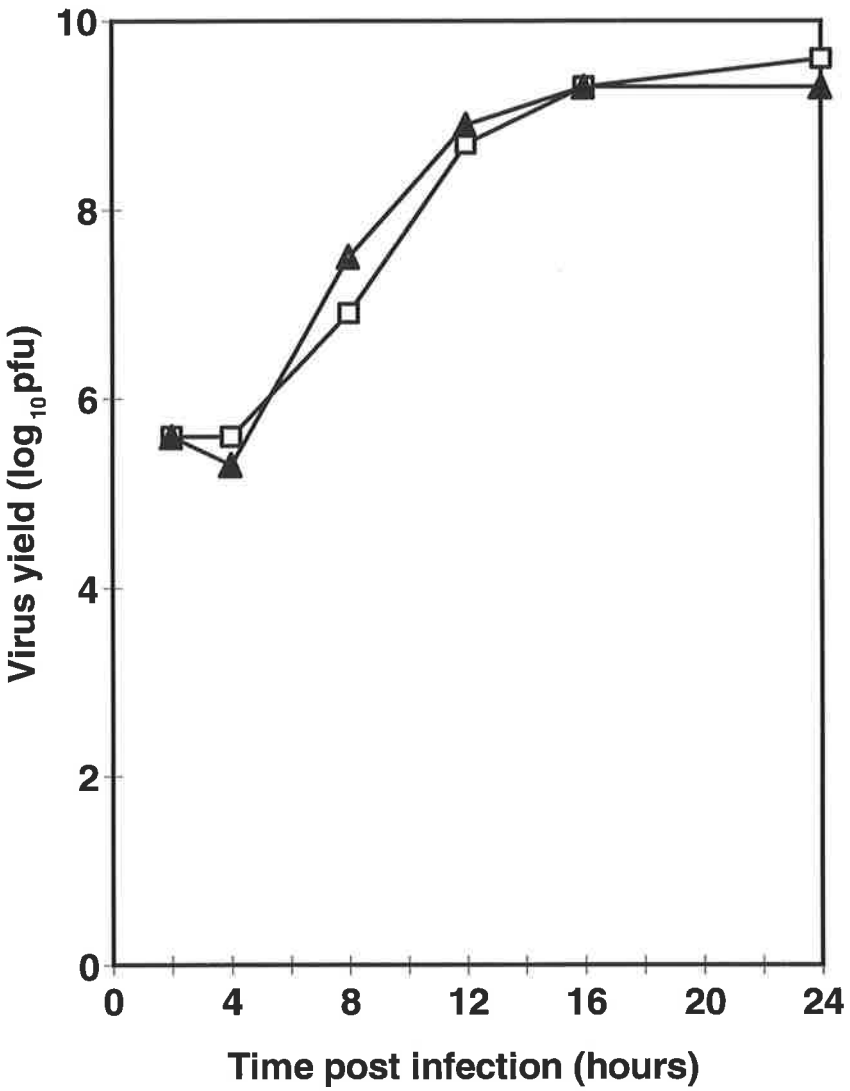
3.1 Growth characteristics of HSV-1 strain S-130 *in vitro*

Single-step growth curves were done to determine whether the H-2K^d recombinant virus S-130 had the same replication kinetics *in vitro* as it's parent virus, C3b. Confluent Vero cell monolayers were infected at a multiplicity of 5 pfu/cell with S-130 or C3b. The amount of infectious virus was quantified from cells harvested 2, 4, 8, 12, 16 and 24 hours after infection (Figure 3.1).

The lag periods, growth rates and virus yields were the same for S-130 and C3b (Figure 3.1). Further, the plaque morphologies of both viruses were identical. It was concluded that S-130 replicates in Vero cells in a manner indistinguishable from its parental virus, C3b.

Figure 3.1

Single-step growth curves for S-130 and C3b in Vero cells. Confluent Vero cell monolayers were infected at a multiplicity of 5 pfu/cell with S-130 (open squares) or C3b (solid triangles). At the indicated times, cells were harvested and infectious virus quantitated by plaque assay. Virus yield was expressed as $\log_{10}$ pfu.


10
8
6
4
2
0
Virus yield (log_{10}pfu)
0
4
8
12
16
20
24
Time post infection (hours)

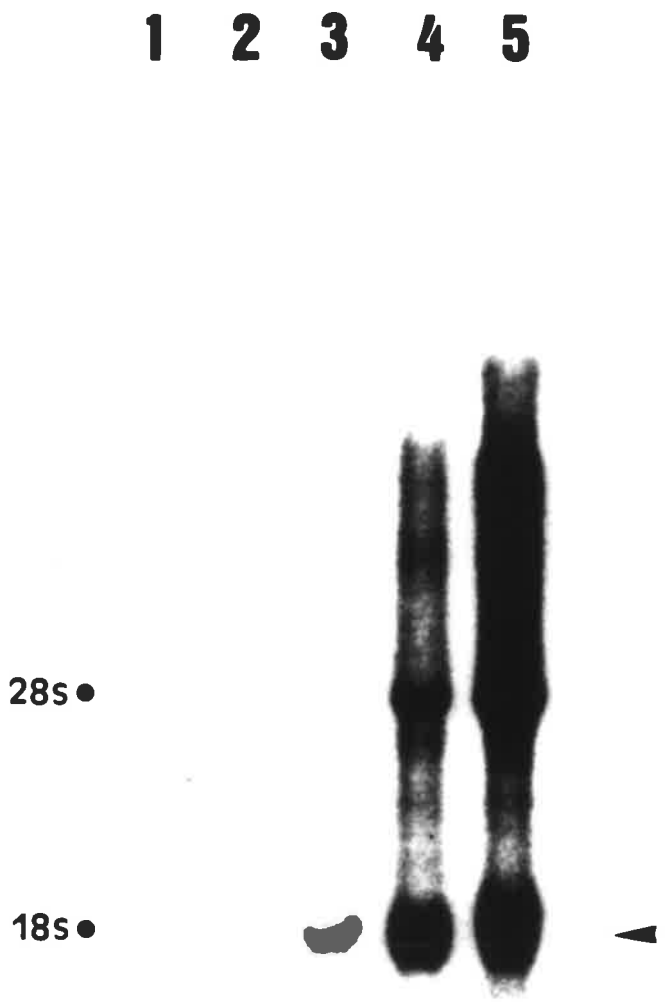
3.2 Detection of RNA sequences encoded by the H-2K^d expression cassette in S-130 infected Vero cells

To show that the H-2K^d expression cassette is transcribed in S-130 infected Vero cells, confluent Vero cell monolayers were infected with S-130 or C3b at a multiplicity of 10 pfu/cell. Total RNA extracted 2.5, 5 or 8 hours after infection was then analysed by northern blot hybridization using a ^{32}P-labelled probe derived from pGEMAA-C (see methods Figure 2.2).

A 1.8kb RNA species, corresponding to the expected migration of full length H-2K^d transcripts from the H-2K^d expression cassette was detected at all three timepoints (Figure 3.2). The intensity of the 1.8kb band increased over the time period studied. Larger, less abundant RNA transcripts were detected in RNA extracted 5 and 8 hours after infection. This was thought to be a result of inefficient transcriptional termination at the BgH poly A site, because 'read through' transcription is common late in the viral replicative cycle. As expected, the probe used in this study did not hybridize to RNA extracted from uninfected or C3b infected Vero cells. It was concluded that the H-2K^d expression cassette is transcribed in S-130 infected cells *in vitro*.

Figure 3.2

Detection of H-2K^d RNA by northern blot hybridization and autoradiography (8 hour exposure). RNA samples (5μg) were electrophoresed, transferred to nitrocellulose and hybridized to a ^{32}P-labelled DNA probe derived from pGEMAA-C, washed and exposed to Kodak X-AR x-ray film. Lanes 1 and 2 contain RNA samples from uninfected Vero and C3b infected Vero cells (8 hours after infection), respectively. Lanes 3 to 5 contain RNA samples from S-130 infected Vero cells 2.5, 5 and 8 hours after infection, respectively. Arrowhead indicates the position of the 1.8kb H-2K^d RNA species derived from the H-2K^d expression cassette. Positions of the 1.9kb 18s and 4.8kb 28s ribosomal RNAs are indicated.


1 2 3 4 5
28s
18s

3.3 Detection of H-2K^d antigens in S-130 infected Vero, L929 and IMR-32 cells

3.3.1 Immunohistochemical studies

H-2K^d antigen expression was studied in three cell lines, namely Vero, L929 and IMR-32 cells. Veros are African green monkey kidney cells and L929s are mouse fibroblast cells. IMR-32 is a human neuroblastoma cell line that expresses extremely low levels of αC and β_2m mRNA and cell-surface MHC-Ia molecules (Marozzi *et al.*, 1993).

Confluent Vero, L929 and IMR-32 cell monolayers were infected at a multiplicity of 10 pfu/cell with S-130 or C3b, harvested 5 hours after infection and tested immunohistochemically for H-2K^d antigens using an anti-H-2K$^{d/b}$/D^d monoclonal antibody, 34-1-2S. P815 cells, which constitutively express H-2K^d antigens were included as a positive control. To ensure that staining of S-130 infected cells was H-2K^d specific, three controls were included: (i) uninfected cells were reacted with 34-1-2S, (ii) C3b infected cells were reacted with 34-1-2S and (iii) S-130 infected cells were reacted with a monoclonal antibody (ID4.5) raised against a salmonella antigen.

H-2K^d staining was detected only in S-130 infected Vero, L929 and IMR-32 cells and in uninfected P815 cells. Figure 3.3 A shows typical staining for H-2K^d antigens in S-130 infected IMR-32 cells. No staining was seen in C3b infected or uninfected IMR-32 cells (Figure 3.3 B & 3.3 C respectively). S-130 infected IMR-32 cells did not stain with ID4.5 (Figure 3.3 D). Staining for H-2K^d antigens was seen in P815 cells (Figure 3.3 E). It was concluded that H-2K^d antigens derived from the expression cassette of S-130 are expressed in a variety of cell lines, including a human neuroblastoma cell line, IMR-32, which expresses extremely low levels of MHC-Ia molecules.

3.3.2 Detection of cell-surface H-2K^d antigens by rosetting

A rosetting assay was used to determine whether H-2K^d antigens are expressed on the surfaces of S-130 infected cells. Rosetting was chosen because it is a very sensitive and simple method for the detection of cell-surface molecules (Goding, 1976 b; Parish & McKenzie, 1978).

Confluent Vero and IMR-32 cells were infected at a multiplicity of 10 pfu/cell with S-130 or C3b and harvested 5 hours after infection. Cell suspensions were reacted with 34-1-2S (anti-H-2K$^{d/b}$/D^d) coated SRBC. P815 cells were included as a control for the presence of cell-surface H-2K^d antigens. Uninfected Vero or IMR-32 cells and ID4.5 coated SRBC were used to determine the extent of non-specific rosetting. 34-1-2S coated SRBC formed rosettes with cells expressing H-2K^d antigens on their surfaces (Figure 3.4). The proportion of

Figure 3.3

Photomicrographs showing detection of H-2K^{d} antigens by immunohistochemistry (brown staining). Specific staining was detected in (A) IMR-32 cells infected with S-130 and (E) P815 cells reacted with 34-1-2S. Specific staining was not detected in (B) IMR-32 cells infected with C3b or (C) uninfected IMR-32 cells reacted with 34-1-2S. (D) S-130 infected IMR-32 cells did not stain with ID4.5. Cells were lightly counterstained with rapid haematoxylin. Magnification is x 960 (A-E).

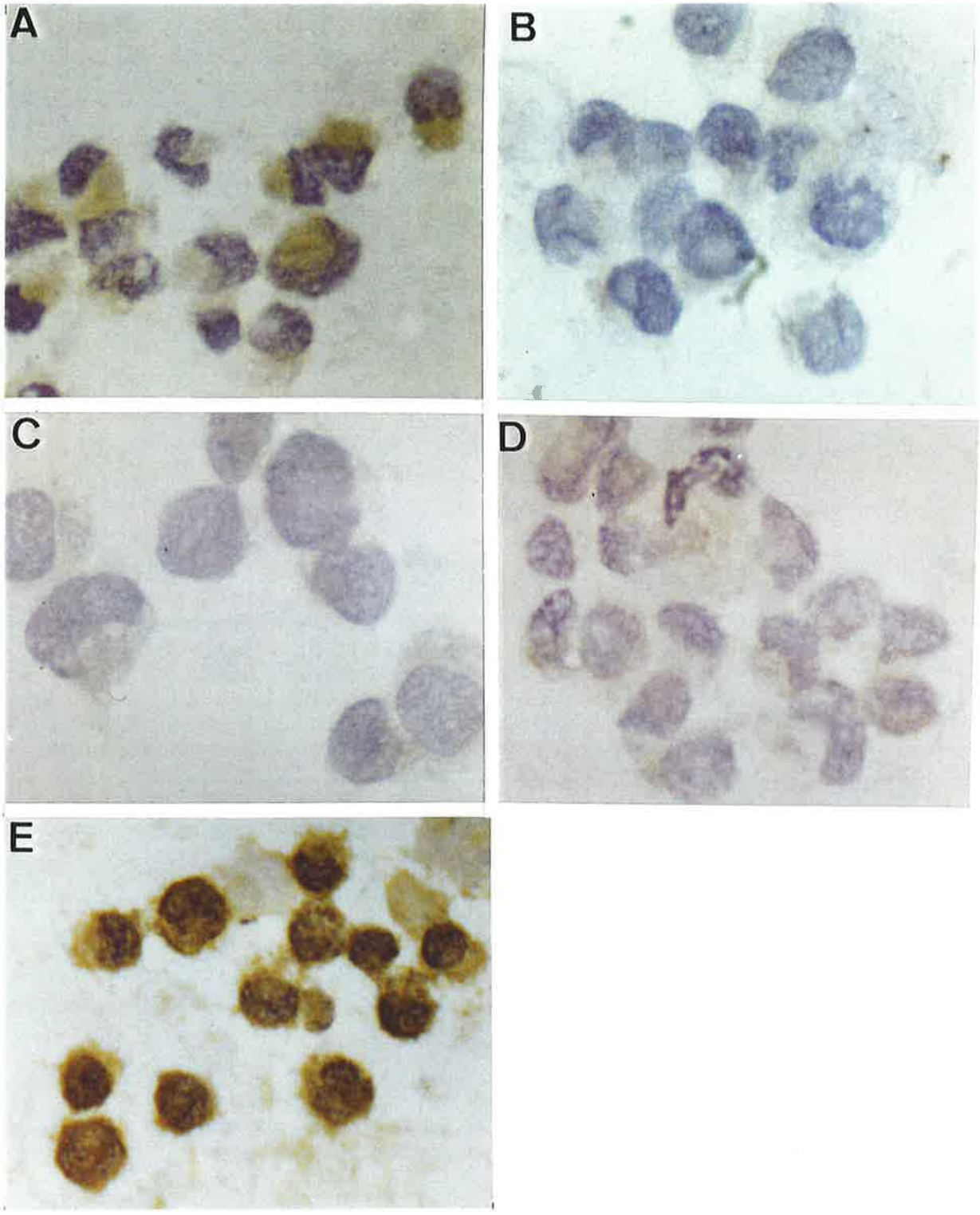
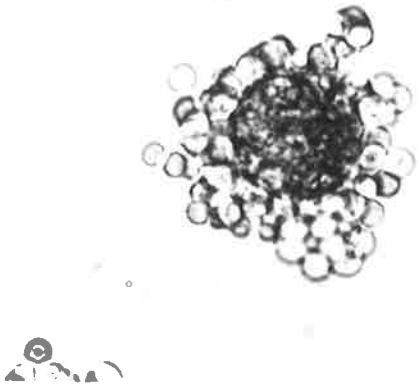

A
B
C
D
E

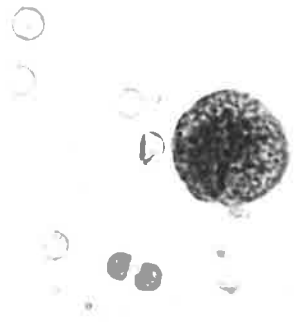
Figure 3.4

Photomicrographs showing detection of cell-surface H-2K^d antigens by rosetting. 34-1-2S reacted with (A) IMR-32 cells from S-130 infected cultures showing a typical rosette comprised of a large IMR-32 cell surrounded by smaller SRBC and (E) P815 cells. (B) Typical view of an IMR-32 cell from C3b infected cultures and (C) an uninfected IMR-32 cell reacted with 34-1-2S. (D) ID4.5 reacted with IMR-32 cells from S-130 infected cultures. Cells were counterstained with methyl violet. Magnification is x 960 (A-E).

A.



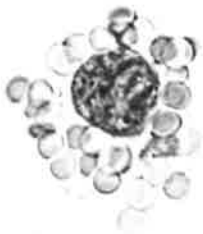
B.



C.

D.

E.



cells infected with S-130 or C3b was determined by an infectious centre assay.

32% of Vero and 30% of IMR-32 cells in S-130 infected cultures formed rosettes with 34-1-2S coated SRBC (Table 3.1). In contrast, the percentages of rosette forming cells (RFC) in C3b infected Vero and IMR-32 cultures was 0.13% and 0.58%, respectively. By infectious centre assay it was shown that 80% of Vero and 44% of IMR-32 cells were infected with S-130 and 86% of Vero and 36% of IMR-32 cells were infected with C3b. From these data it was calculated that 40% of S-130 infected Vero cells and 68% of S-130 infected IMR-32 cells expressed H-2K^d antigens on their surfaces. It was concluded that a high proportion of cells infected with S-130 *in vitro* expressed H-2K^d antigens on their surfaces.

3.4 Detection of H-2K^d and β_2m on the surfaces of S-130 infected K562 cells

K562 cells are MHC-Ia deficient human myelogenous leukemia cells (Lozzio & Lozzio, 1979) which express β_2m but not αCs (Sutherland *et al.,* 1985; Chen *et al.,* 1986). Without αC, β_2m cannot be transported to the cell-surface. Therefore, K562 cells were used to determine whether S-130 derived H-2K^d αCs were expressed in association with β_2m on the cell-surface during S-130 infection. A rosetting assay was used to detect cell-surface β_2m and H-2K^d

Table 3.1 Detection of Surface H-2K^d Antigen Expression by Rosetting on Cell Lines Infected with S-130

HSV Strain	Cell Line	Antibody	Number RFC*/Total Counted (%)
S-130	Vero	34-1-2S	237/740 (32)
		ID4.5	1/700 (0.14)
	IMR-32	34-1-2S	122/413 (30)
		ID4.5	1/690 (0.15)
C3b	Vero	34-1-2S	1/788 (0.13)
		ID4.5	3/850 (0.35)
	IMR-32	34-1-2S	4/690 (0.58)
		ID4.5	2/800 (0.25)
Uninfected	Vero	34-1-2S	1/800 (0.13)
		ID4.5	2/750 (0.27)
	IMR-32	34-1-2S	1/760 (0.13)
		ID4.5	2/892 (0.22)
Uninfected	P815	34-1-2S	267/354 (75)
		ID4.5	3/750 (0.40)

* Rosette Forming Cells

antigens.

K562 cells were infected at a multiplicity of 10 pfu/cell with S-130 or C3b and harvested 5 hours after infection. Surface β_2m and H-2K^d antigen expression was detected using anti-human β_2m (7B6C) and anti-H-2K$^{d/b}$/D^d (34-1-2S) monoclonal antibodies, respectively. In these studies HUT 78 (a human lymphoblastoid cell line) and P815 cells were included as controls for the presence of cell-surface human β_2m and H-2K^d antigen, respectively. In addition, uninfected cells and a monoclonal antibody ID4.5 were included to determine the extent of non-β_2m and non-H-2K^d specific rosetting.

β_2m was not specifically detected on uninfected K562 cells, confirming that it cannot be expressed at the cell-surface in the absence of αCs (Table 3.2). However, 51.1% of cells from S-130 infected cultures expressed H-2K^d on their surfaces and 47.8% expressed β_2m. β_2m was not specifically detected on the surfaces of C3b infected cells. It was concluded that β_2m is transported to the surfaces of S-130 infected K562 cells in association with H-2K^d.

Table 3.2 Detection of H-2K^d and β_2m on the Surface of S-130 Infected K562 Cells by Rosetting

HSV Strain	Cell Line	Antibody	Number RFC*/Total Counted (%)
S-130	K562	7B6C 34-1-2S ID4.5	332/694 (47.8) 388/760 (51.1) 10/866 (1.2)
C3b	K562	7B6C 34-1-2S ID4.5	16/872 (1.8) 7/897 (0.8) 13/838 (1.5)
Uninfected	K562	7B6C 34-1-2S ID4.5	12/915 (1.3) 0/1080 (0) 0/718 (0)
Uninfected	P815	34-1-2S	250/250 (100)
Uninfected	HUT-78	7B6C	238/262 (90.8)

* Rosette Forming Cells

3.5 Analysis of the susceptibility of S-130 infected target cells to lysis by anti-H-2^d specific cytotoxic T cells

MHC class I antigens are potent alloantigens (transplantation antigens) which, when expressed on the surfaces of cells, are recognized by alloreactive CTLs. To determine whether S-130 encoded H-$2K^d$ is recognized as an alloantigen, the ability of anti-H-2^d specific CTLs generated *in vitro* by mixed lymphocyte culture (MLC), to lyse S-130 infected Vero and L929 cells was assessed.

The conditions for the generation of anti-H-2^d CTLs in MLC were optimized by varying responder:stimulator ratios and IL-2 concentration. C57BL10 (H-2^b) responder spleen cells were cultured *in vitro* with irradiated P815 (H-2^d) stimulator cells at responder:stimulator ratios of 4:1 and 10:1 with 5, 10 or 20 units of IL-2. After 5 days in culture, resulting effector cells were harvested and tested for CTL activity against ^{51}Cr labelled P815 and L929 cells (Table 3.3). Depending on the conditions used, anti-H-2^d specific CTLs lysed P815 cells with varying efficiency. On the basis of these results, cultures containing 5 units of IL-2 and a responder:stimulator ratio of 10:1 were chosen for generating anti-H-2^d specific CTLs.

Table 3.3 Capacity of P815 Cells to Stimulate Anti-H-2^d Specific CTLs in 5 Day MLC

Responder (C57Bl10 Spleen Cells):Stimulator (P815) Ratio	Units Of IL-2	Effector:Target Ratio	% ^{51}Cr Release From Target Cells ±SEM	
			P815	L929
4:1	10	40:1 20:1 10:1 5:1	83 ±0.1 70 ±1.1 72 ±1.5 55 ±4.7	21 ±2.0 12 ±3.3 7 ±3.2 4 ±1.5
10:1	5	40:1 20:1 10:1 5:1	85 ±4.0 73 ±4.9 74 ±3.1 70 ±27	30 ±4.7 17 ±0.9 9 ±2.0 4 ±2.9
10:1	10	40:1 20:1 10:1 5:1	52 ±2.4 52 ±2.6 49 ±11.1 67 ±2.3	36 ±7.4 25 ±10 16 ±4.4 10 ±1.4
10:1	20	40:1 20:1 10:1 5:1	44 ±11.8 42 ±4.0 55 ±3.0 56 ±3.0	28 ±4.9 24 ±5.9 17 ±2.0 8 ±8.1

To show that L929 (H-2^k) cells were capable of stimulating anti-H-2^k specific CTLs in MLC the following experiment was done. Anti-H-2^k CTLs were generated *in vitro* as described in section 2.2.17, using L929 (H-2^k) cells as stimulators in the presence or absence of IL-2. As expected, anti-H-2^k specific CTLs generated in the presence of IL-2 were able to specifically lyse L929 cells (Table 3.4).

Anti-H-2^d specific CTLs generated using the culture conditions described on the previous page, were tested for cytotoxic activity against ^{51}Cr labelled (i) uninfected P815 and L929 cells and S-130 or C3b infected L929 cells (Figure 3.5 A) and (ii) uninfected P815 and Vero cells and S-130 or C3b infected Vero cells (Figure 3.5 B). Anti-H-2^d specific CTLs specifically lysed P815 cells as expected but did not lyse L929 or Vero cells whether or not they were infected with S-130 (Figure 3.5 A & B, respectively). It was concluded that anti-H-2^d specific CTLs raised by stimulating H-2^b spleen cells with P815 cells did not lyse S-130 infected L929 or Vero cells.

3.6 Summary

S-130 was shown to replicate in a manner indistinguishable from its parental virus strain C3b in Vero cells and to synthesize full length H-$2K^d$ RNA transcripts. Immunohistochemical studies showed that H-$2K^d$ antigens were synthesized in S-130 infected Vero and L929 cells and a human neuroblastoma

Table 3.4 Capacity of L929 Cells to Stimulate Anti-H-2^k Specific CTLs in 5 Day MLC

Responder (C57Bl10 Spleen Cells):Stimulator (L929) Ratio	Units Of IL-2	Effector:Target Ratio	% ^{51}Cr Release From Target Cells ±SEM	
			P815	L929
10:1	10	20:1	15 ±2.0	59 ±2.0
		10:1	6 ±6.0	30 ±5.0
		5:1	10 ±3.4	18 ±4.5
		2.5:1	3 ±5.0	8 ±2.0
10:1	0	20:1	-2.0 ±6.0	7 ±1.0
		10:1	-2.7 ±1.0	2 ±8.0
		5:1	-1.8 ±2.0	1 ±0.3
		2.5:1	-2.3 ±4.0	0.4±2.0

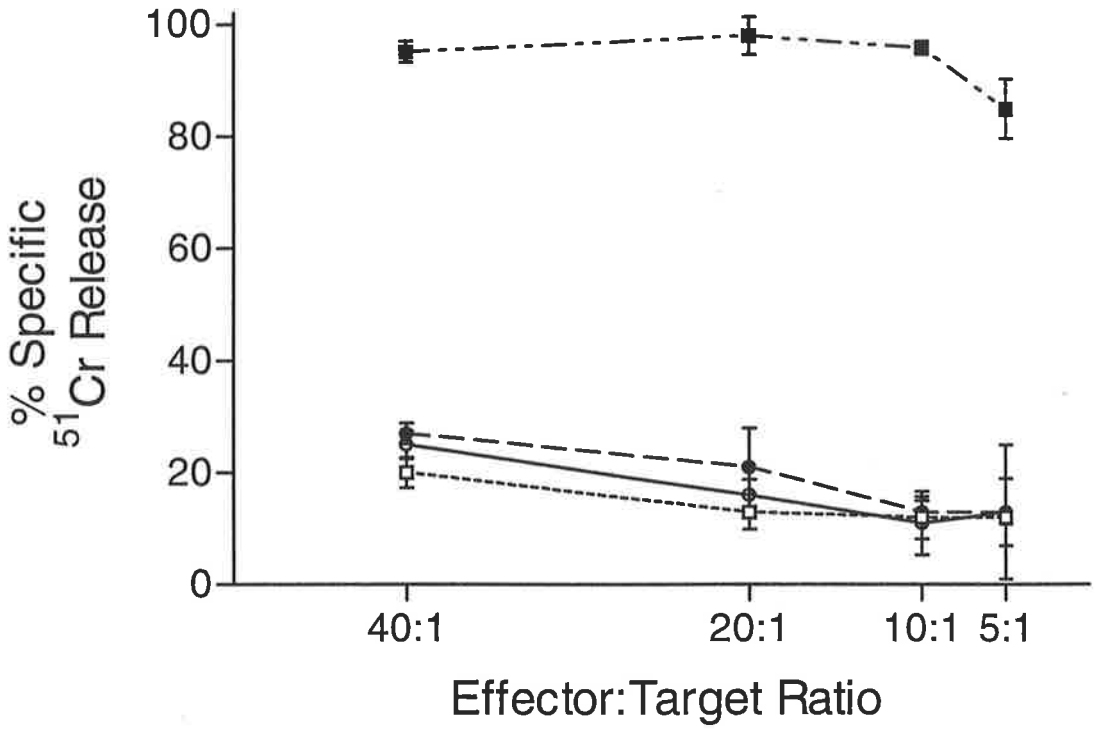
Figure 3.5

Analysis of the susceptibility of S-130 infected target cells to lysis by anti-H-2^d specific CTLs. Assays were done using ^{51}Cr labelled target cells and anti-H-2^d specific CTLs. The latter were generated by culturing C57BL10 (H-2^b) spleen cells *in vitro* with irradiated P815 (H-2^d) cells. Data presented are the means of triplicate assays ± standard error of the mean.

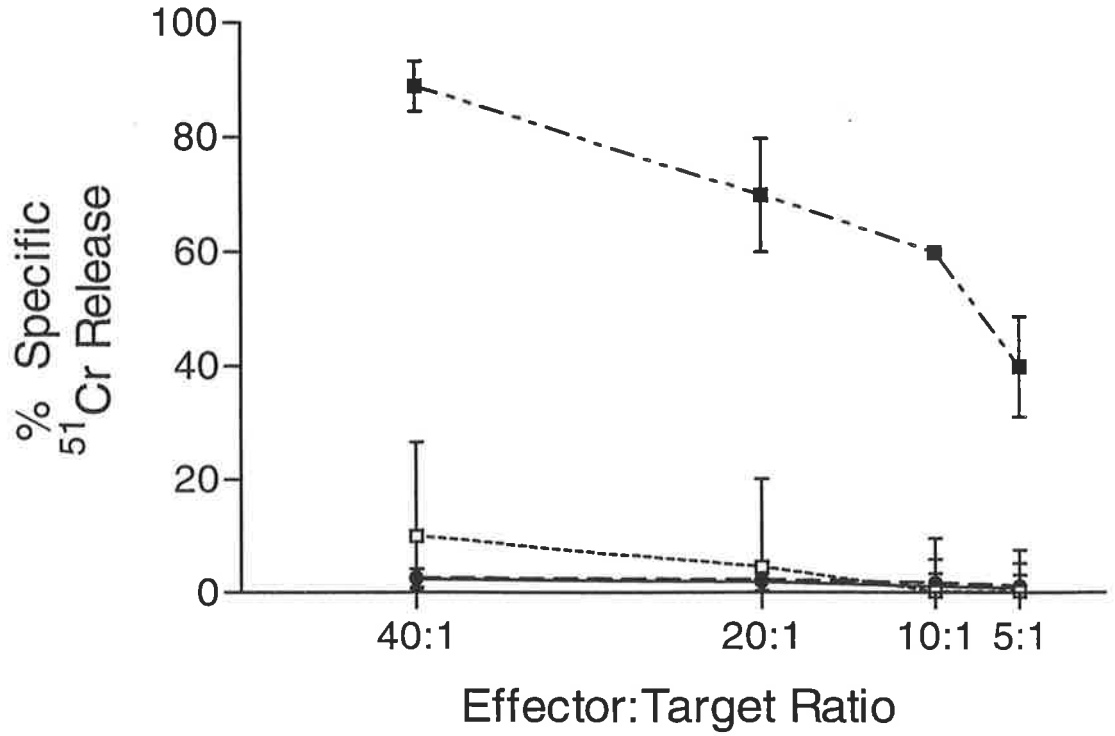
(A) L929 target cells infected with S-130 (closed circles) or C3b (open circles). P815 (closed squares) and uninfected L929 (open squares) target cells were included as positive and negative controls, respectively, for H-2^d specific lysis.

(B) Vero target cells infected with S-130 (closed circles) or C3b (open circles). P815 (closed squares) and uninfected Vero (open squares) target cells were included as positive and negative controls, respectively, for H-2^d specific lysis.

A.


100
80
60
40
20
0
% Specific
^{51}Cr Release
40:1
20:1
10:1
5:1
Effector:Target Ratio

B.


100
80
60
40
20
0
% Specific
^{51}Cr Release
40:1
20:1
10:1
5:1
Effector:Target Ratio

cell line that expresses low levels of cell-surface MHC-Ia molecules. Further, H-2K^d antigens were detected on the surfaces of S-130 infected Vero and IMR-32 cells (L929 cells were not tested). αC deficient K562 cells expressed β_2m at the cell-surface after infection with S-130. This result suggested that H-2K^d αCs were expressed at the cell-surface in association with β_2m. S-130 infected Vero and L929 (H-2^k) cells were not lysed by anti-H-2^d alloreactive CTLs generated by stimulation of H-2^b spleen cells with P815 (H-2^d) cells.

4. CHARACTERIZATION OF HSV-1 STRAIN S-130 *IN VIVO* USING A MOUSE MODEL

Based on the data presented in the previous chapter, it was hypothesized that H-2K^d antigens would be expressed on the surfaces of S-130 infected primary sensory neurons *in vivo*.

A well characterized mouse model (Simmons & Nash, 1984), which makes use of the segmental sensory innervation of flank skin, was used to address this hypothesis. In mice, the thoracic region of the PNS is divided into 13 structural segments (T1-T13), which innervate bilaterally symmetrical bands of skin called dermatomes. Virus is inoculated into flank skin in the eighth thoracic dermatome (T8) from where it travels along peripheral nerves to invade the corresponding thoracic ganglion (Speck & Simmons, 1991; Slobedman *et al.,* 1994). In this system, productive ganglionic infection, quantified by the presence of infectious virus, viral mRNA and viral antigen positive neurons has been shown previously to peak 5 days after inoculation of flank skin and be rapidly terminated over the next two days (Speck & Simmons, 1991). Subsequently, viral DNA is sequestered in a latent, non-replicating state in a proportion of neurons. On the basis of these data, ganglia were analysed 5 and 11-24 days after flank inoculation for evidence of H-2K^d expression associated with productive and latent viral infection, respectively.

The specific aims of the experiments described in this chapter were to show that:

(i) S-130 is neuroinvasive (ie. targets PNS neurons) following cutaneous inoculation.

(ii) the H-2K^d expression cassette of S-130 is transcribed in productively infected ganglia.

(iii) S-130 infected primary sensory neurons synthesize H-2K^d antigens.

(iv) H-2K^d is expressed on the surfaces of S-130 infected neurons.

(v) S-130 establishes latent infection.

(vi) the H-2K^d expression cassette remains active during latency.

4.1 Neuroinvasiveness of S-130 in mice

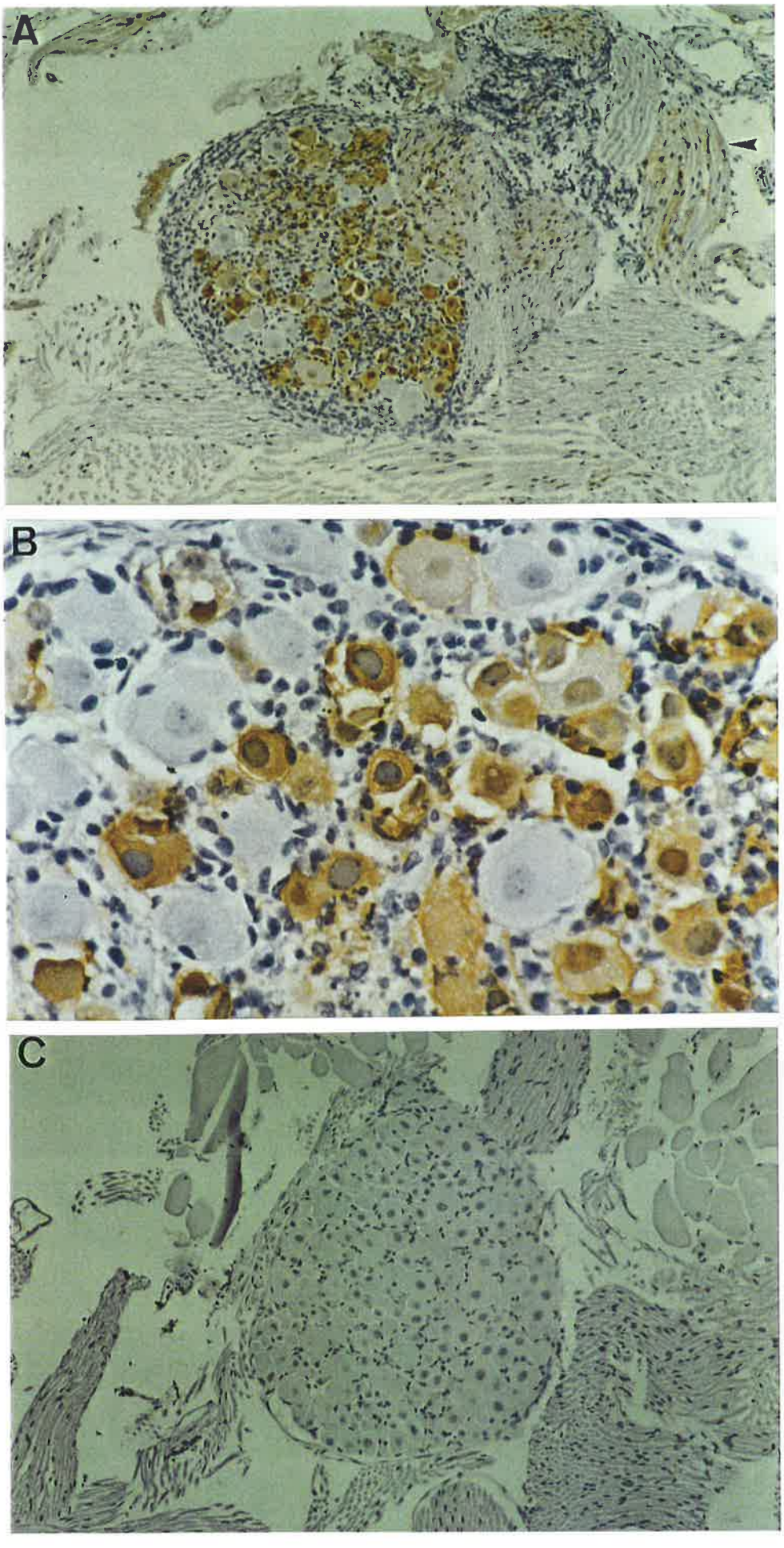
To determine whether S-130 is neuroinvasive, thoracic ganglia were tested five days after inoculation of virus into flank skin for the presence of viral antigens, using immunohistochemistry. A non-H-2K^d mouse strain, C3H/HEJ (H-2^k) was used because in subsequent experiments this mouse strain was considered suitable for immunohistochemical studies of H-2K^d antigen expression by S-130 infected cells.

Sections (5μm) of PLP fixed ganglia were tested for HSV antigens using a polyclonal rabbit antiserum against HSV-1 infected cells. Sections from uninfected C3H/HEJ mice were included in all immunohistochemical reactions as a control for non-specific staining.

Several randomly selected sections of each paraffin embedded block of infected tissue (each section containing approximately 10 ganglionic profiles) were used to determine the mean number of HSV antigen positive neuronal profiles per ganglionic profile. In C3H/HEJ mice productively infected with S-130 or C3b, HSV viral antigens were detected in neuronal cell bodies and Schwann cells in nerve fibre axons (Figure 4.1 A & B). The proportion of viral antigen positive neuronal profiles in S-130 infected ganglionic tissue (15.8%) was similiar to that in tissue infected with the parental strain C3b (14.7%). No staining was detected in uninfected ganglionic tissue (Figure 4.1

Figure 4.1

Photomicrographs of C3H/HEJ mouse ganglionic sections showing detection of HSV antigens by immunohistochemistry (brown staining). Specific staining was detected in spinal ganglia productively infected with (A) S-130 and (B) C3b. Arrowhead shows nerve fibre staining in Schwann cells. Specific staining was not detected in (C) uninfected C3H/HEJ mouse ganglionic sections. Sections were counterstained with rapid haematoxylin. Magnification is x 75 (A & C) and x 384 (B).


A
B
C

C). It was concluded that S-130 infects neurons of the peripheral nervous system following cutaneous inoculation.

4.2 Detection of RNA sequences encoded by the H-2K^d expression cassette in spinal ganglia productively infected with S-130

To determine whether the H-2K^d expression cassette of S-130 is transcribed in productively infected ganglia, synthesis of H-2K^d RNA in ganglia infected with S-130 was assessed by (i) *in situ* hybridization and (ii) northern blot hybridization.

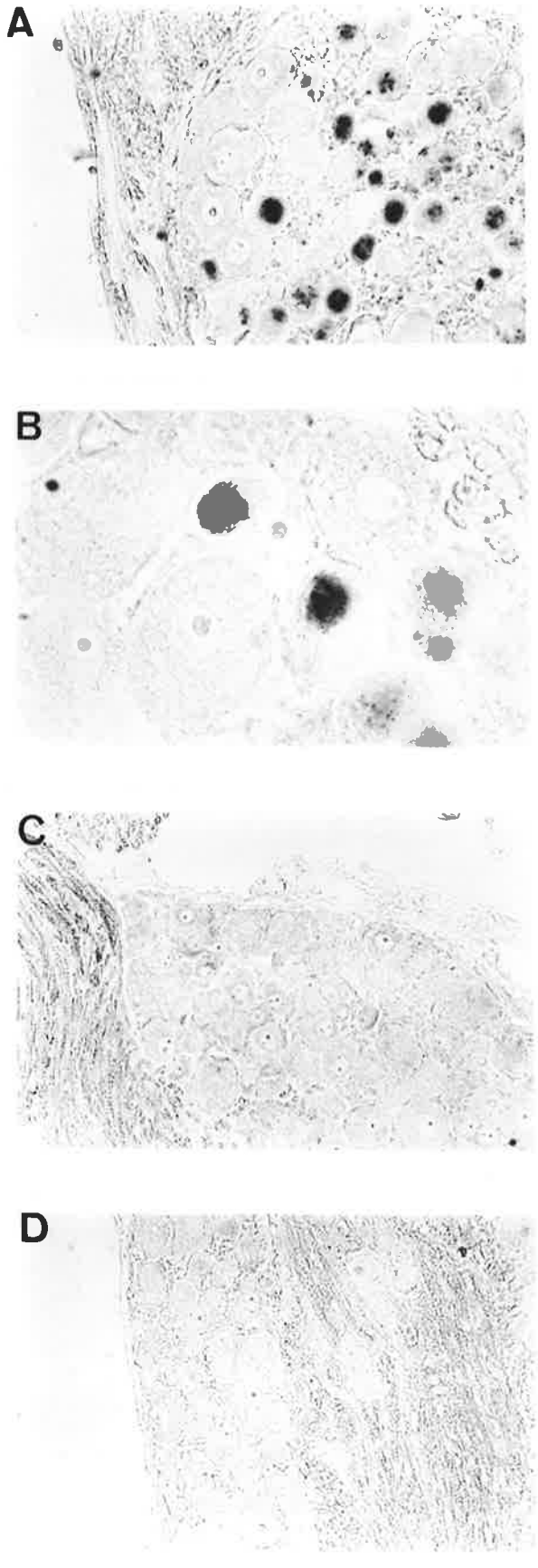
4.2.1 *In situ* hybridization studies

A high resolution, non-isotopic *in situ* hybridization (ISH) protocol was used to detect and localize viral nucleic acid sequences in spinal ganglia, 5 days after infection with S-130 or C3b.

Sections (5μm) of PLP fixed ganglia from S-130 and C3b infected mice (10 mice/group) were hybridized with a strand specific DIG-labelled riboprobe (generated from pGEMAA-C), anti-sense to and specific for RNA transcripts derived from the S-130 H-2K^d expression cassette. Sections from uninfected animals were included in all ISH experiments as a control for non-specific hybridization. The probe hybridized strongly with ganglionic sections from S-130 infected mice (Figure 4.2 A & B). Staining was confined to neuronal

Figure 4.2

Photomicrographs showing detection and localization of S-130 specific viral nucleic acid sequences by non-isotopic *in situ* hybridization (black areas). Ganglionic sections from C3H/HEJ mice were hybridized to a DIG-labelled riboprobe generated from the T7 promoter of pGEMAA-C. (A & B) positive hybridization in neuronal nuclei of ganglionic sections from C3H/HEJ mice productively infected with S-130. Specific hybridization was not detected in ganglionic sections from (C) C3H/HEJ mice productively infected with C3b or (D) from uninfected C3H/HEJ mice. Magnification is x 96 (A, C & D) and x 384 (B).

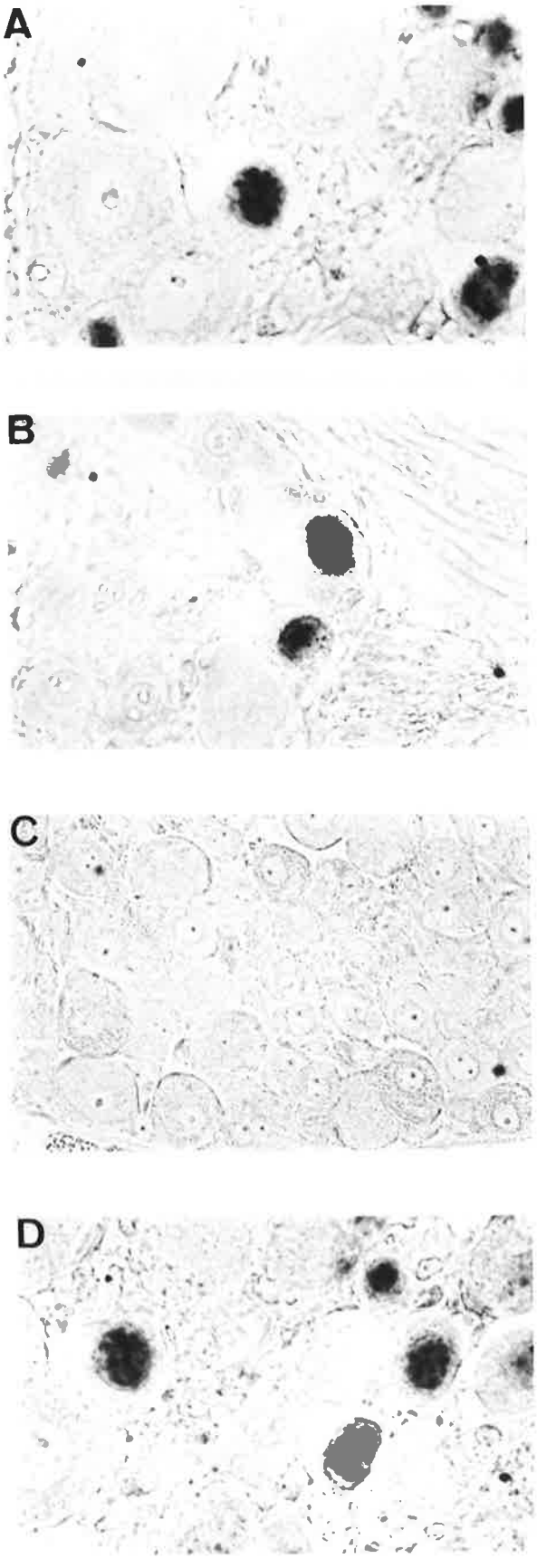

A
B
C
D

nuclei and appeared as sharply defined foci or was diffuse, sometimes filling the entire nucleus. Probe did not hybridize with sections from C3b infected or uninfected mice (Figure 4.2 C & D, respectively). It was concluded that nucleic acid sequences derived from the H-2K^d expression cassette were present in detectable amounts in neurons of S-130 infected sensory ganglia. These data confirm the conclusions of the immunohistochemical studies (section 4.1) showing that S-130 is neuroinvasive.

To determine whether the probe was detecting RNA, DNA sequences or both, S-130 infected ganglionic sections were treated with DNAse or RNAse prior to hybridization. Pretreatment with DNAse completely abolished staining by the probe (Figure 4.3 C), but digestion of sections with RNAse did not detectably alter the intensity of probe binding (Figure 4.3 B). It was concluded that 5 days after infection the nucleic acid sequences detected by the probe were DNA rather than RNA. Riboprobes of the same sense as RNA transcripts derived from the H-2K^d expression cassette of S-130 (generated from pBSAA-R), were used to confirm this conclusion. The probe hybridized strongly to neuronal nuclei in S-130 infected ganglionic sections (Figure 4.3 D) and staining was indistinguishable in intensity from that seen with the anti-sense pGEMAA-C derived riboprobe (Figure 4.3 A). Thus, S-130 was shown to be a suitable vector for delivery of a H-2K^d gene into neurons, but ISH failed to detect H-2K^d RNA transcripts derived from the H-2K^d expression cassette.

Figure 4.3

Determination of S-130 specific viral nucleic acid type by *in situ* hybridization. Ganglionic sections from C3H/HEJ mice productively infected with S-130 were (A) untreated, (B) digested with RNAse or (C) digested with DNAse before hybridization to a DIG-labelled riboprobe generated from the T7 promoter of pGEMAA-C. (D) ganglionic sections were also hybridized to a probe generated from the T7 promoter of pBSAA-R, which produces transcripts that are the same sense as RNA transcripts derived from the H-$2K^d$ expression cassette. Note that hybridization to S-130 derived H-$2K^d$ nucleic acids (black areas) was detected only in untreated and RNAse treated sections probed with pGEMAA-C and untreated sections probed with pBSAA-R. Hybridization signal was abolished in DNAse treated sections probed with pGEMAA-C. Photomicrograph magnification is x 300 (A, B & D) and x 200 (C).


A
B
C
D

4.2.2 Northern blot hybridization

The preceding *in situ* hybridization studies failed to detect S-130 derived H-2K^d mRNA transcription. Transcripts were therefore sought by northern blot analysis of RNA extracted from ganglia of S-130 infected mice.

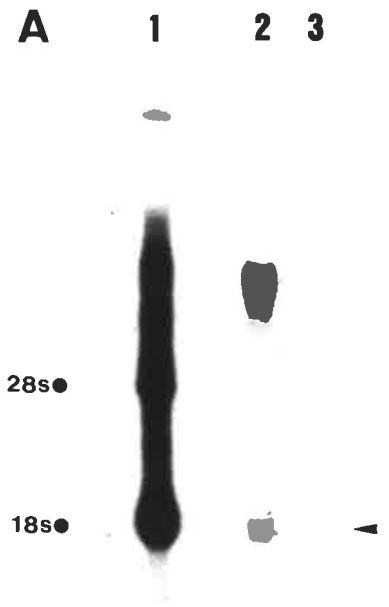
Twenty adult C3H/HEJ (H-2^k) mice were infected (day 0) with S-130 (10 mice) or C3b (10 mice). On day 5, RNA samples (5μg) from spinal ganglia were analysed by northern blot hybridization using a ^{32}P-labelled DNA probe generated from pGEMAA-C, which contains sequences present in the H-2K^d expression cassette (see methods Figure 2.2) but not host MHC-Ia. RNA extracted from Vero cells 5 hours after infection with S-130 was included as a positive control for transcripts derived from the H-2K^d expression cassette.

In RNA extracted from S-130 infected ganglia and S-130 infected Vero cells the probe detected a RNA species, corresponding to the expected migration (~1.8kb) of H-2K^d transcripts derived from the H-2K^d expression cassette (Figure 4.4 A). The probe also hybridized with a less abundant transcript of ~5.0kb, consistent with inefficient transcriptional termination at the BgH poly A site. A higher molecular weight smear observed in the S-130 infected RNA sample was consistent with the presence of H-2K^d DNA. Probe did not hybridize to RNA extracted from C3b infected mice. It was concluded that S-130 synthesizes H-2K^d RNA derived from the H-2K^d expression cassette in sensory ganglia of productively infected mice.

Figure 4.4

(A) Detection of H-2K^d RNA by northern blot hybridization and autoradiography (7 hour exposure). RNA samples (5μg) were electrophoresed, transferred to nitrocellulose and hybridized to a ^{32}P-labelled DNA probe generated from pGEMAA-C, washed and exposed to Kodak X-AR x-ray film. Lane 1 contains RNA from Vero cells 5 hours after infection with S-130. Lanes 2 and 3 contain RNA from spinal ganglia of C3H/HEJ mice productively infected with S-130 and C3b, respectively. Arrowhead indicates the position of the 1.8kb H-2K^d RNA species derived from the H-2K^d expression cassette of S-130. Positions of the 1.9kb 18s and 4.8kb 28s ribosomal RNAs are indicated.

(B) Reprobe of (A) with a ^{32}P-labelled DNA probe derived from pBS4 (64 hour exposure to X-AR film). Arrowhead indicates the position of the 4.3kb ICP4 RNA species.


A
1
2
3
28s
18s


B
1
2
3

To validate the use of C3b as a negative control in this experiment, it was necessary to show that S-130 and C3b replicated to equivalent levels in spinal ganglia. To address this issue, the nitrocellulose filter was reprobed for a HSV-1 IE gene transcript, using a ^{32}P-labelled DNA probe generated from pBS-4, which contains 1.8kb of the gene encoding ICP4. In S-130 and C3b infected ganglionic RNA samples, and in the S-130 infected Vero cell RNA sample, probe hybridized with a transcript of ~4.3kb, corresponding to the expected migration of ICP4 mRNA (Figure 4.4 B). The autoradiographic intensities of the 4.3kb bands in S-130 and C3b infected ganglionic RNA were comparable, consistent with similiar levels of viral replication in S-130 and C3b infected spinal ganglia.

4.3 Detection of H-2K^d antigen synthesis by S-130 infected ganglionic neurons

To determine whether S-130 infected primary sensory neurons synthesize H-2K^d antigens, S-130 infected ganglia were analysed by (i) immunohistochemistry, to detect cytoplasmic antigen and (ii) rosetting, to detect cell-surface antigen.

4.3.1 Immunohistochemical studies

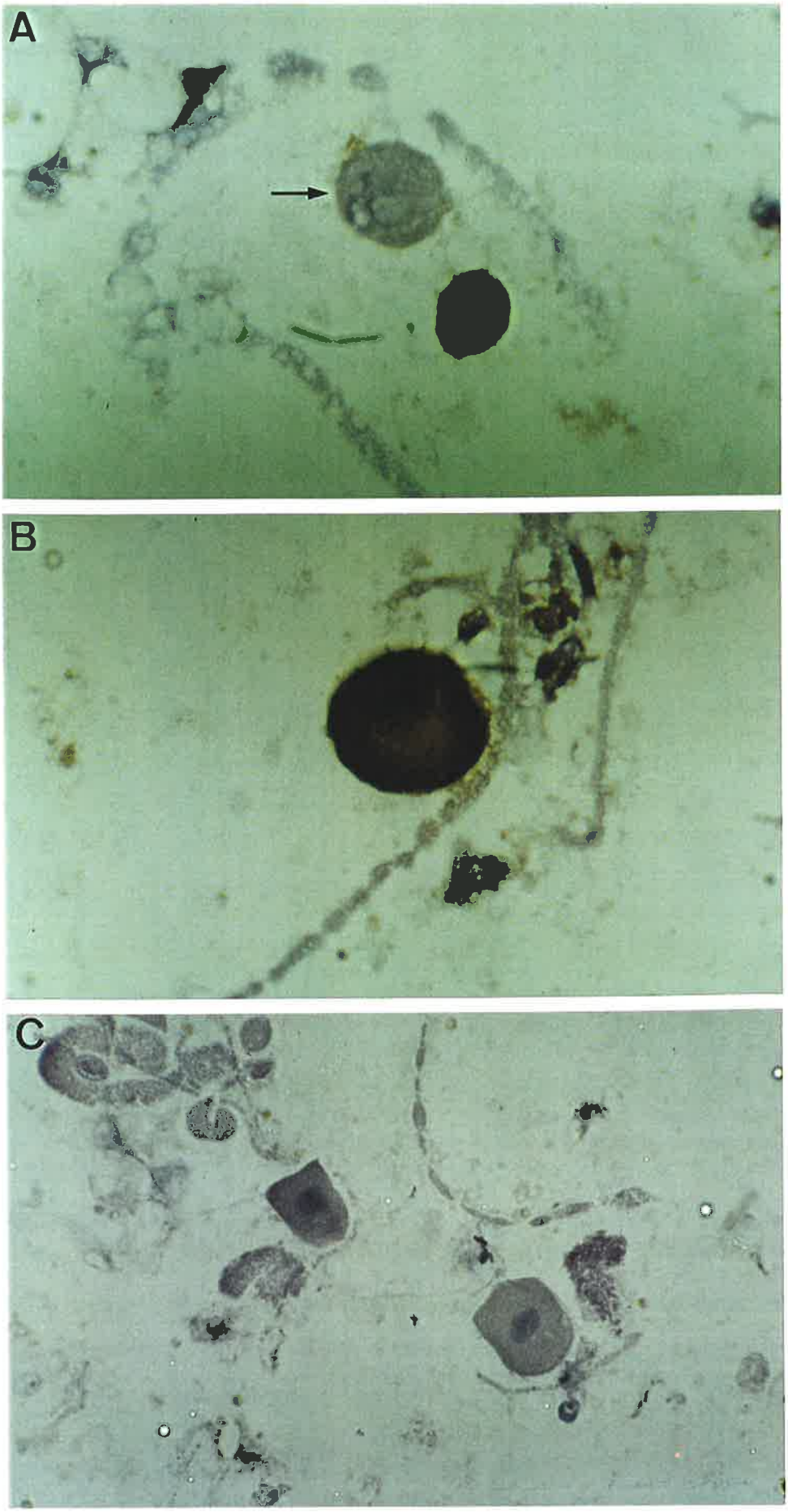
For immunohistochemical examination of H-2K^d antigen expression, frozen ganglionic sections from mice productively infected with S-130 were used, on the basis that several groups have detected MHC-Ia antigens in frozen sections of nervous system tissue (Maehlen *et al.,* 1989; Gogate *et al.,* 1991; Pereira *et al.,* 1994). However, attempts to detect H-2K^d antigens in frozen sections were unsuccessful in this study. This problem was overcome by studying cell smears prepared by enzymatic dissociation of ganglia.

The feasibility of using dissociated ganglionic preparations in such a study was assessed by immunohistochemical staining for viral antigens rather than H-2K^d. Twenty adult C3H/HEJ mice were infected (day 0) with S-130 (10 mice) or C3b (10 mice). Cell smears prepared from spinal ganglia 5 days after infection were tested immunohistochemically for HSV viral antigens. In addition, dissociated ganglionic cells from uninfected mice were used as a control for the specificity of staining. On the basis of their large size (>20μm diameter) and characteristic morphology, primary sensory neurons in cell smears could be readily distinguished from glial cells.

HSV viral antigen positive neurons were detected in smears made from S-130 and C3b infected C3H/HEJ ganglia (Figure 4.5 A & B, respectively). HSV viral antigens were also detected in glial cells (not illustrated). No staining was detected in neurons or any other cell type dissociated from uninfected ganglia

Figure 4.5

Photomicrographs showing detection of HSV antigens by immunohistochemistry (brown staining). Specific staining was detected in dissociated ganglionic neurons from C3H/HEJ mice productively infected with (A) S-130 or (B) C3b. Specific staining was not detected in (C) uninfected C3H/HEJ mouse ganglionic neurons. Arrow shows a viral antigen negative neuron. Cell smears were lightly counterstained with rapid haematoxylin. Magnification is x 300 (A & C) and x 384 (B).


A
B
C

(Figure 4.5 C). The number of viral antigen positive neurons in S-130 and C3b dissociated ganglia 5 days after infection was similiar (Table 4.1). These data suggested that enzymatic dissociation maintained the integrity of HSV infected neurons and therefore such preparations could be used in further immunohistochemical analyses.

In order to detect H-2K^d antigens, cell smears were reacted with monoclonal antibody 34-1-2S (anti-H-2K$^{d/b}$/D^d). P815 cells, which constitutively express H-2K^d, were included in all reactions as a positive control. ID4.5, a monoclonal antibody against a salmonella antigen and of the same isotype as 34-1-2S was included in all reactions to control for non-specific binding of primary antibody. H-2^k mice were selected for this experiment in order to ensure that 34-1-2S detected MHC-Ia molecules derived from S-130, rather than from host cells.

Twenty adult C3H/HEJ mice were divided into two experimental groups and infected (day 0) with S-130 (10 mice) or C3b (10 mice). Spinal ganglia were removed and cell smears were prepared 5 days after infection.

34-1-2S reacted with neurons from S-130 infected ganglia (Figure 4.6 A) and P815 cells (positive control; not illustrated). To determine the proportion of neurons expressing H-2K^d antigens, approximately eight hundred neurons were counted (Table 4.2). No staining was seen in neurons of C3b infected or

Table 4.1 Detection of HSV Antigens in Productively Infected Neurons

HSV Strain	Number Viral Antigen +ve Neurons/Total Counted	% Viral Antigen +ve neurons
C3b	105/1014	10.4
S-130	126/1242	10.1

Figure 4.6

Photomicrographs showing detection of H-2K^{d} antigens by immunohistochemistry (brown staining). (A) specific staining with 34-1-2S in dissociated ganglionic neurons from C3H/HEJ mice productively infected with S-130. Specific staining was not detected in dissociated ganglionic neurons from (B) C3H/HEJ mice productively infected with C3b or (C) uninfected C3H/HEJ mice reacted with 34-1-2S. (D) ganglionic neurons from C3H/HEJ mice productively infected with S-130 did not stain with ID4.5. Cell smears were lightly counterstained with rapid haematoxylin. Magnification is x 384 (A-D).

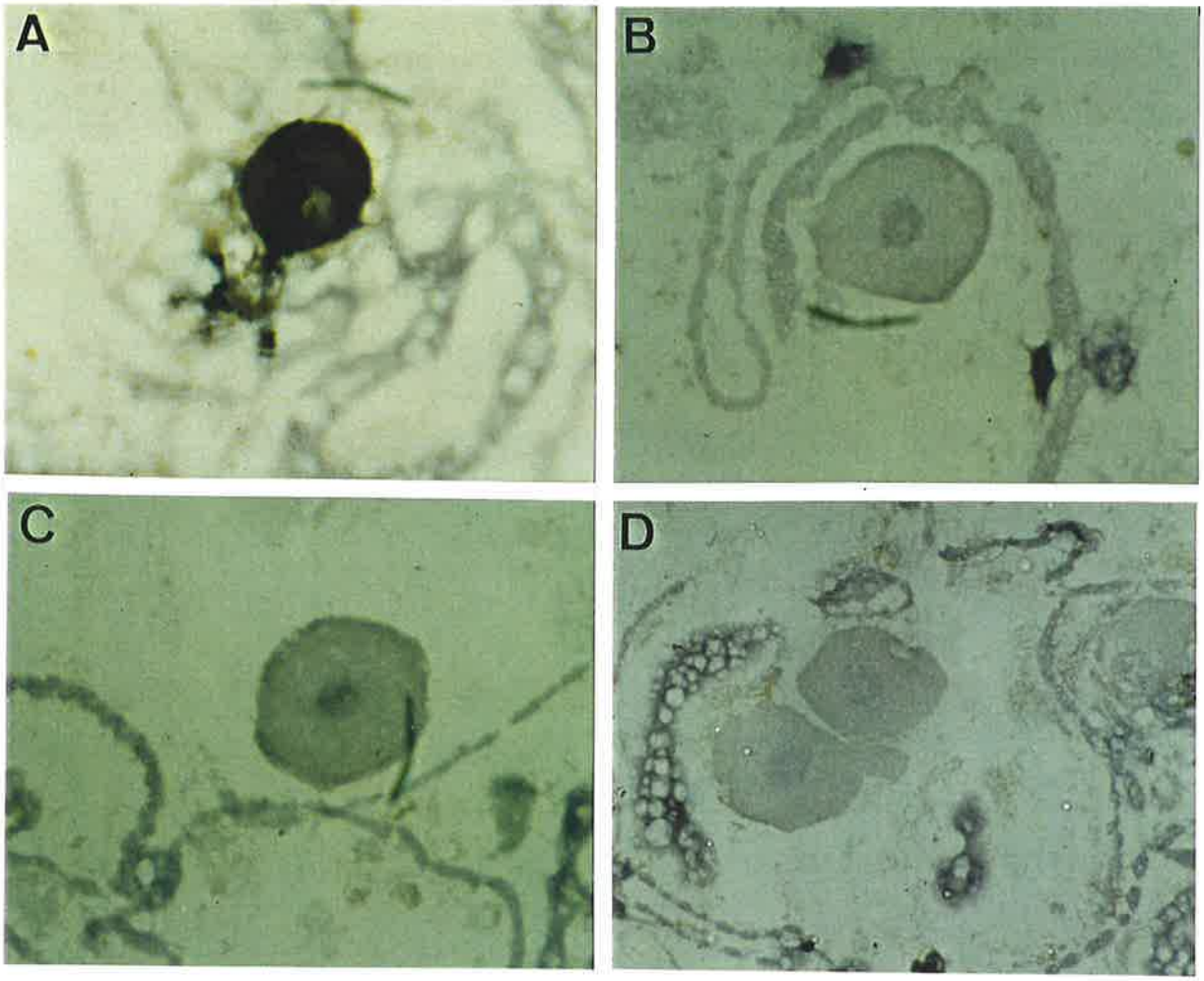

A
B
C
D

Table 4.2 Detection of H-2K^d Antigens in Primary Sensory Neurons 5 Days After Infection of C3H/HEJ Mice

Cell Type	Antibody	Number H-2K^d +ve Cells/Total Counted (%)	Number Viral Antigen +ve Neurons/Total Counted (%)
Neurons From S-130 Infected Ganglia	34-1-2S	32/866 (3.7)	130/818 (15.9)
	ID4.5	0/850 (0)	
Neurons From C3b Infected Ganglia (Negative Control)	34-1-2S	0/840 (0)	128/800 (16.0)
	ID4.5	0/812 (0)	
Uninfected P815 (Positive Control)	34-1-2S	212/212 (100)	N/A
	ID4.5	0/200 (0)	

N/A Not Applicable

uninfected ganglia (Figure 4.6 B & C, respectively). S-130 infected ganglionic neurons did not stain with ID4.5 (Figure 4.6 D). To show that mice infected with S-130 and C3b had comparable levels of neuronal infection, the number of viral antigen positive neurons was compared and found to be similiar for both viruses (Table 4.2). Viral antigens were detected in a greater proportion of neurons than H-2K^d.

4.3.2 Rosetting assays

A major aim of this project was to determine whether HSV infected neurons are able to express MHC-Ia molecules at their surfaces *in vivo*. S-130 was constructed to address this aim.

A rosetting assay was used to detect cell-surface H-2K^d antigens. Initially, a direct rosetting technique, using 34-1-2S (anti-H-2K$^{d/b}$D^b) coated SRBC, was used (Parish & McKenzie, 1978). Several attempts using this procedure yielded suboptimal results in that only ~70% of P815 cells, which constitutively express H-2K^d antigens, formed rosettes and no neuronal rosettes were detected from S-130 infected ganglia. To increase the sensitivity of rosetting, an indirect rosetting procedure using protein A coupled SRBC (prot A-SRBC) was used. This procedure has been shown to be more sensitive than direct coupling of antibody to SRBC and more sensitive than antibody and complement mediated cytotoxic assays for detection of cell-surface MHC-Ia molecules (Sandrin *et al.,* 1978). When prot A-SRBC were used in the assay, H-2K^d molecules were

detected on at least 95% of P815 cells.

To study H-2K^d antigen expression in spinal ganglia, two groups of ten adult C3H/HEJ mice were infected (day 0) with S-130 or C3b and cell suspensions from pooled spinal ganglia were prepared on day 5. Cells dissociated from uninfected ganglia were included to determine the extent of non-specific rosetting. The number of viral antigen positive neurons in the S-130 and C3b infected cell preparations was determined immunohistochemically.

Rosette forming cells (RFC) were detected frequently in S-130 infected preparations (eg. Figure 4.7 A & B) but rarely in preparations from C3b infected (eg. Figure 4.7 C) or uninfected mice (eg. Figure 4.7 E & F) (Table 4.3). To verify that ganglia infected with S-130 and C3b had a comparable level of viral infection, the number of viral antigen positive neurons in the two experimental groups were compared and found to be similiar (Table 4.3). Further, the proportion of viral antigen positive cells from S-130 infected ganglia was similiar to the proportion of RFC. It was concluded that the majority of mouse sensory ganglionic neurons infected with S-130 *in vivo* express H-2K^d antigens on their surfaces.

Figure 4.7

Photomicrographs showing detection of cell-surface H-2K^d antigens by rosetting. (A & B) 34-1-2S reacted with dissociated ganglionic neurons from C3H/HEJ mice productively infected with S-130 showing a typical rosette, arrowed, comprised of a large primary sensory neuron surrounded by smaller SRBC. Arrowheads indicate non-rosetted neurons. Rosettes were rarely detected on ganglionic neurons from (C) C3H/HEJ mice productively infected with C3b or (E & F) uninfected C3H/HEJ mice reacted with 34-1-2S. In similar experiments, (D) rosettes were rarely detected on ganglionic neurons from C3H/HEJ mice productively infected with S-130 reacted with ID4.5. Cells were counterstained with methyl violet. Magnification is x 200 (A & E) and x 750 (B, C, D & F).

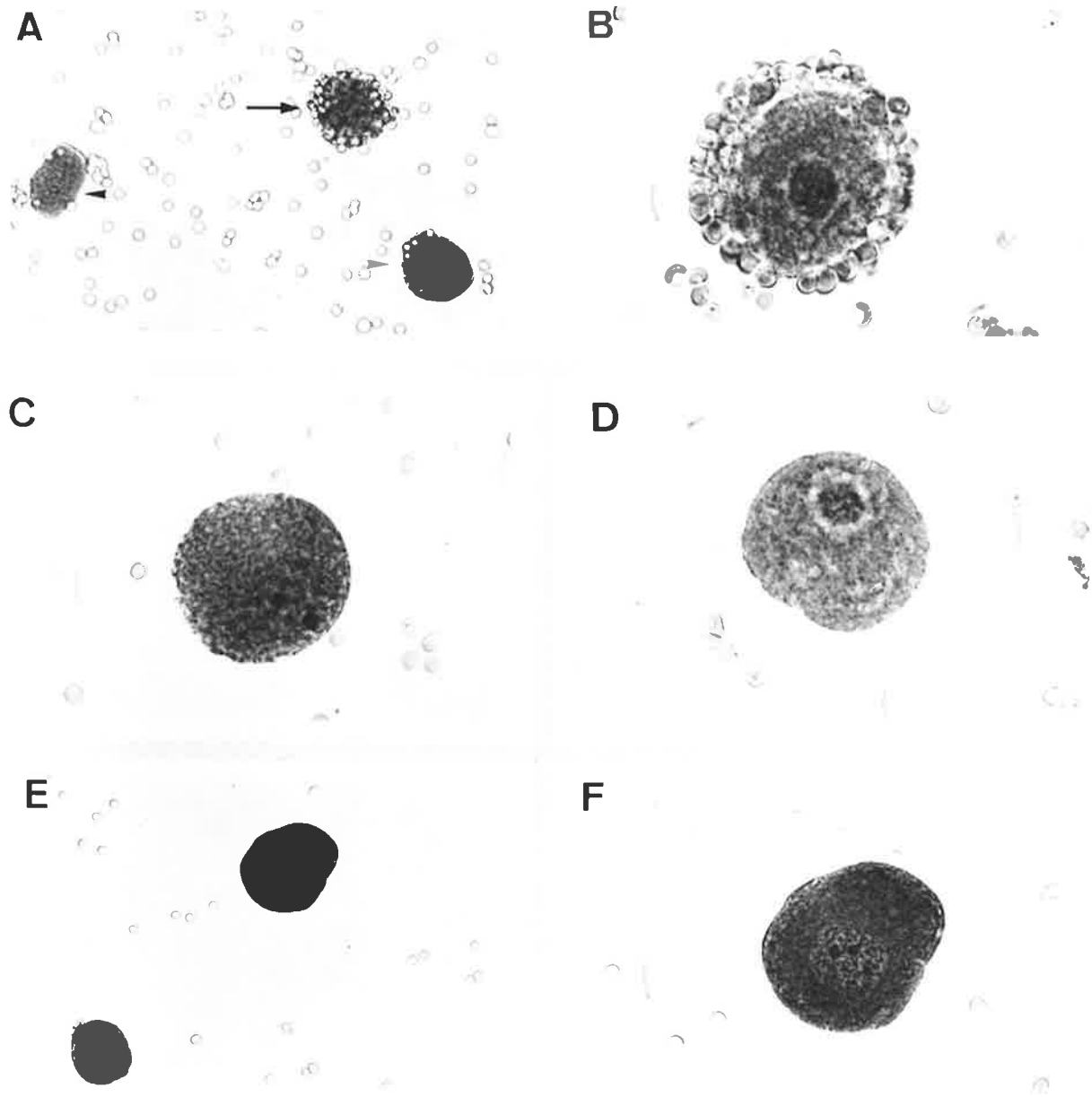

A
B
C
D
E
F

Table 4.3 Detection of Surface H-2K^d Antigens On Primary Sensory Neurons 5 Days After Infection of C3H/HEJ Mice

Cell Type	Antibody	Number RFC*/Total Counted (%)	% Viral Antigen Positive Neurons
Neurons From S-130 Infected Ganglia	34-1-2S	45/790 (5.7)	5.0
Neurons From C3b Infected Ganglia (Negative Control)	34-1-2S	12/636 (1.9)	6.0
Neurons From Uninfected Ganglia (Negative Control)	34-1-2S	9/546 (1.6)	0

* Rosette Forming Cells

4.4 Effect of H-2K^d expression in mice of a different H-2 haplotype on the rate of virus clearance from productively infected skin and spinal ganglia

It was shown previously (see section 4.3.2) that H-2K^d antigens are expressed on the surfaces of S-130 infected mouse ganglionic neurons. It was reasoned that neuronal expression of H-2K^d in mice of a different H-2 haplotype might lead to accelerated clearance of virus from infected ganglia, as a result of enhanced killing of infected neurons. To address this hypothesis, S-130 and C3b infectious virus and DNA levels were compared at various times after flank inoculation of C57BL10 (H-2^b) mice.

4.4.1 Infectious virus

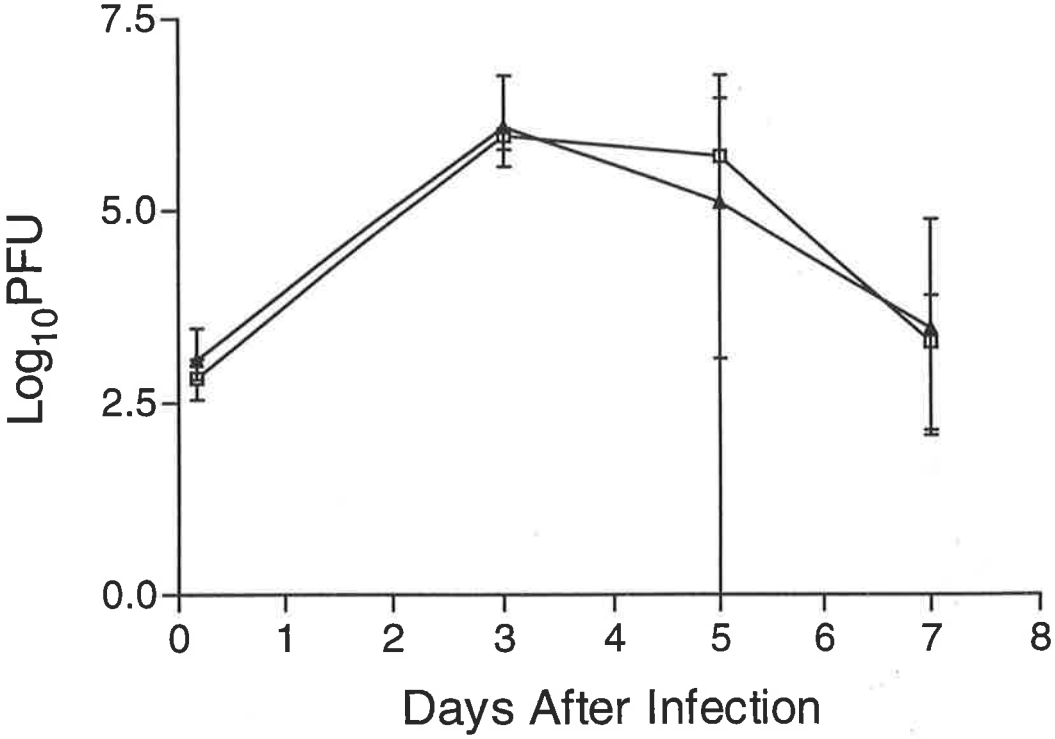
Fifty C57BL10 mice were divided into two experimental groups which were infected (day 0) either with S-130 or C3b. Infectious virus was quantified in homogenates of left flank skin (encompassing the inoculation site) and spinal ganglia, from groups of 5 mice killed 4 hours and 1, 3, 5 and 7 days after inoculation (Figure 4.8).

Four hours after infection, the amounts of virus recovered from S-130 and C3b infected skin samples were not significantly different, indicating that the inocula were equivalent (Figure 4.8 A). Virus recovery from skin peaked 3 days after inoculation and the rate of virus clearance from the skin was similar

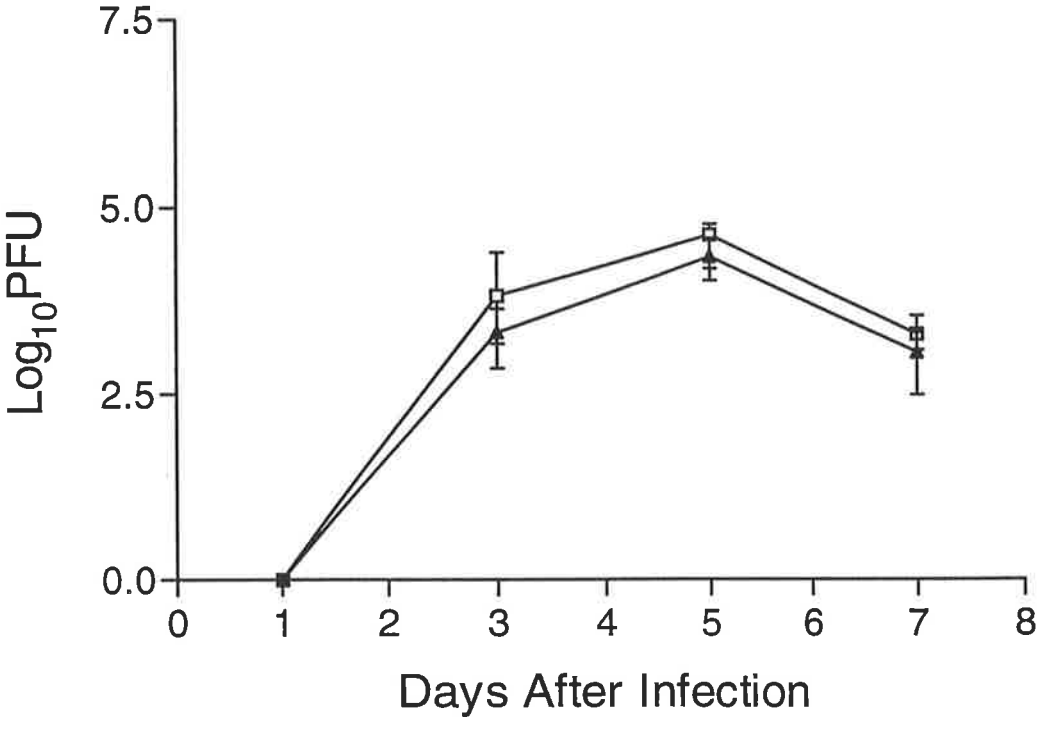
Figure 4.8

Quantification of HSV by plaque assay in homogenates of (A) flank skin and (B) spinal ganglia removed from C57BL10 mice after flank inoculation with S-130 (open squares) or C3b (solid triangles). Virus yield was expressed as $\log_{10}$ pfu. Each point represents the geometric mean titre and range from groups of 5 mice.

A.


7.5
5.0
2.5
0.0
Log10PFU
0
1
2
3
4
5
6
7
8
Days After Infection

B.


7.5
5.0
2.5
0.0
Log10PFU
0
1
2
3
4
5
6
7
8
Days After Infection

for S-130 and C3b. Virus replication in the spinal ganglia peaked 5 days after infection and, again, the amounts of S-130 and C3b recovered at all time points tested was similar (Figure 4.8 B). It was concluded that S-130 and C3b are cleared at the same rate from skin and ganglia of H-2^b mice under the experimental conditions used.

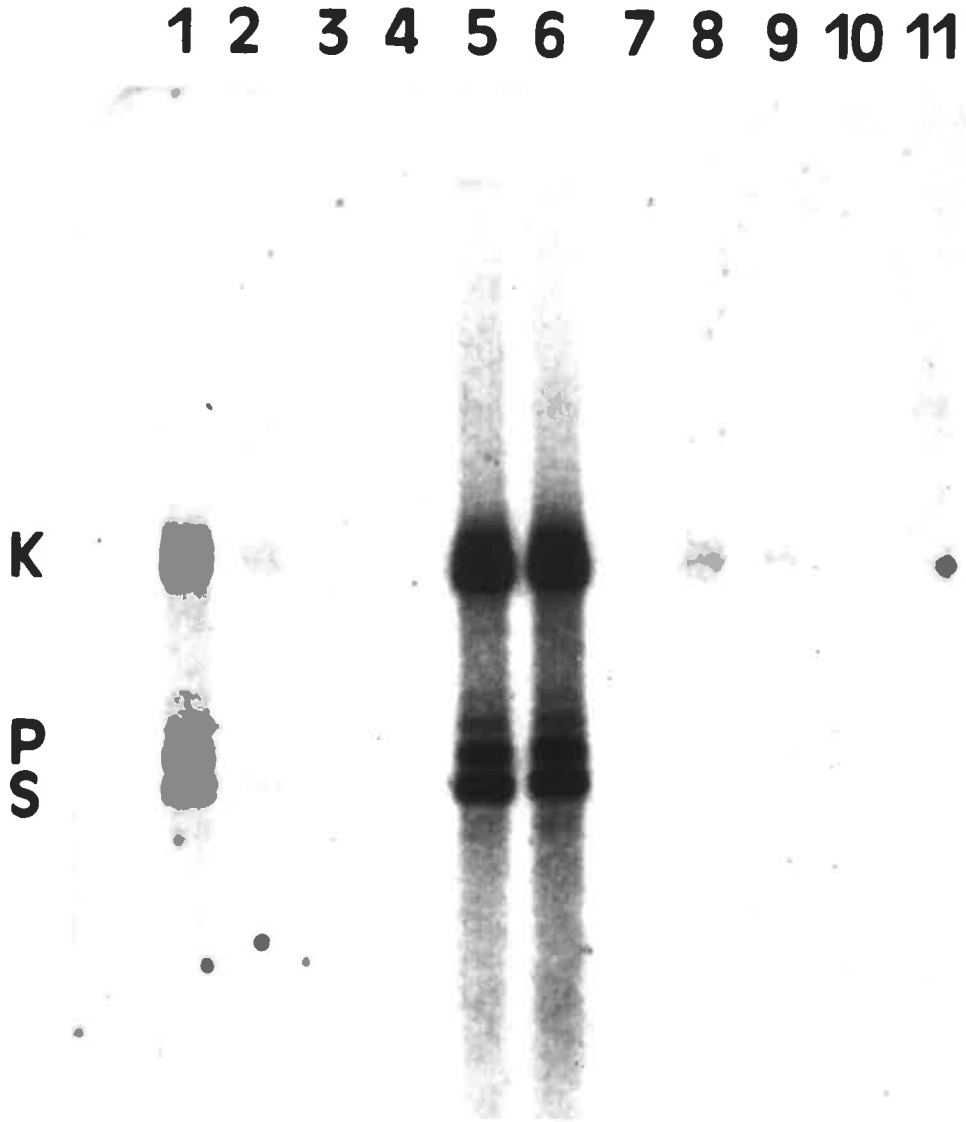
4.4.2 Viral DNA

Groups of 25 C57BL10 mice were infected (day 0) with S-130 or C3b. On days 5 and 11, viral DNA from pooled ganglia was analysed by Southern blot hybridization using a probe spanning the HSV-1 junction region (pBKSP1). For comparison, the amount of infectious virus in ganglia was quantified on day 5 (5 animals per group). Mean titres of $6.3x10^4$ pfu/mouse and $1.5x10^4$ pfu/mouse were recovered from S-130 and C3b infected animals, respectively. Therefore, as in the previous experiment, S-130 and C3b infected C57BL10 mice had similiar levels of productive ganglionic infection on day 5.

Viral genome equivalent "copy per cell" reconstructions were made from SC16 virion DNA. Probe detected three major SC16 *Bam*HI fragments of approximately 5.9kb, 3.6kb and 2.9kb, corresponding to the expected sizes of the *Bam*HI-K (junction), *Bam*HI-P (S, terminal) and *Bam*HI-S (L, terminal) fragments, respectively (Figure 4.9). The intensity of the *Bam*HI-P fragment was slightly less than that of the *Bam*HI-S terminal band. This was expected because the overlap of pBKSP1 with *Bam*HI-S is greater than with *Bam*HI-P

Figure 4.9

Detection of HSV DNA sequences by Southern blotting in spinal ganglia from C57BL10 mice 5 and 11 days after flank inoculation with S-130 (lanes 6 & 9, respectively) or C3b (lanes 5 & 8, respectively). For quantification purposes lanes 1 to 3 represent 5, 0.5 and 0.05 HSV genome copies per cell, respectively. Lane 11 contains uninfected mouse spleen DNA. DNA samples (5μg) were digested with *Bam*HI, transferred to nitrocellulose, hybridized with a ^{32}P-labelled DNA probe generated from pBKSP1 and examined by phosphor imaging (24 hour exposure). The position of the junction (K) and terminal (P and S) fragments are indicated.


1 2 3 4 5 6 7 8 9 10 11
K
P
S

(1.7kb and 0.8kb, respectively). In addition, a ladder of weaker bands which are the result of reiterations of the "*a*" sequence was detected above the terminal fragments.

In *Bam*HI digested ganglionic DNA samples extracted 5 days after infection of mice with S-130 or C3b, the probe also hybridized to junction (*Bam*HI-K) and terminal (*Bam*HI-P & *Bam*HI-S) fragments. On day 11, only the junction region was detected, consistent with the "endless" form of viral DNA associated with latent infection (Rock & Fraser, 1985; Efstathiou *et al.*, 1986). The number of viral genome copies per cell on days 5 and 11 was determined in relation to "copy per cell" reconstructions using ImageQuant software. On day 5 there were 18 HSV genomes per cell in ganglia infected either with S-130 or C3b and on day 11, there were 0.36 and 0.70 HSV genome copies per cell in ganglia infected with S-130 or C3b, respectively. The small copy number difference between S-130 and C3b on day 11 was not considered significant and was most likely attributable to loading less of the S-130 infected DNA sample, based on examination of the ethidium bromide stained gel. The probe did not hybridize to DNA extracted from uninfected mice. The amount of S-130 and C3b DNA recovered 11 days after infection was up to 50 fold-less than that recovered on day 5. It was concluded that rates of clearance of viral DNA from sensory ganglia were the same in S-130 and C3b infected mice.

4.5 Immunohistochemical detection of H-2K^d antigens in ganglionic neurons latently infected with S-130

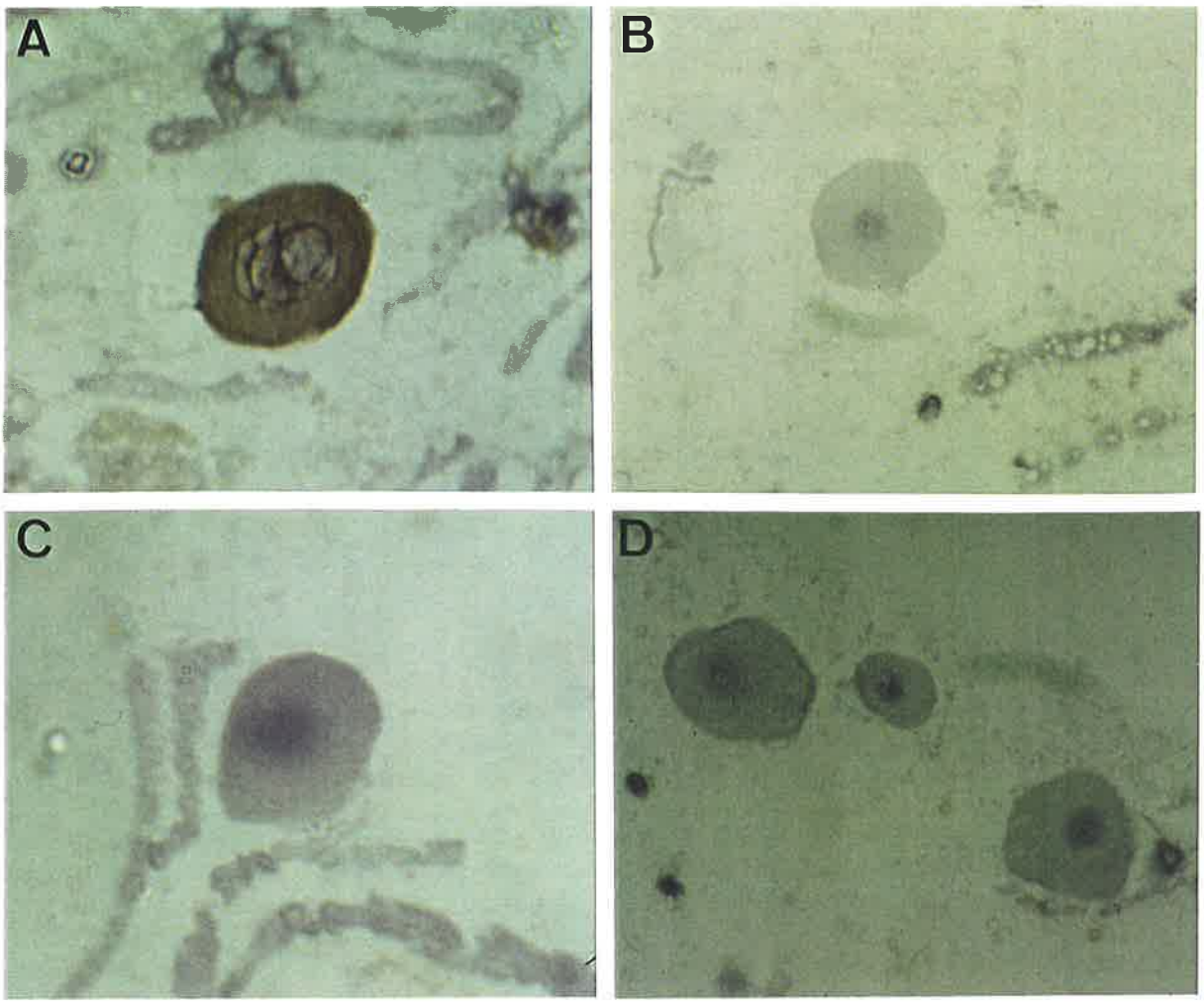
To determine whether the H-2K^d expression cassette remains active during latency, expression of H-2K^d antigens was assessed in sensory neurons from ganglia removed 25 days after infection of mice with S-130.

One group of 10 C3H/HEJ mice was infected (day 0) with S-130 and another group was infected with C3b as a H-2K^d negative control. Ganglia were enzymatically dissociated on day 25 and cells were tested for H-2K^d antigen expression using monoclonal antibody 34-1-2S (anti-H-2K$^{d/b}$/D^d).

H-2K^d antigens were detected in a proportion (<1%) of S-130 infected neurons (Figure 4.10 A). There was no staining of neurons in C3b infected and uninfected mice (Figure 4.10 B & C, respectively). Neurons from S-130 infected mice did not react with the anti-salmonella antibody, ID4.5 (Figure 4.10 D). H-2K^d was readily detected in P815 cells, which constitutively express H-2K^d (not illustrated). It was concluded that H-2K^d antigens are expressed in neurons for at least 25 days after infection with S-130.

Figure 4.10

Photomicrographs showing detection of H-2K^d antigens by immunohistochemistry (brown staining). (A) specific staining with 34-1-2S was detected in dissociated ganglionic neurons from C3H/HEJ mice 25 days after infection with S-130. Specific staining was not detected in dissociated ganglionic neurons from (B) C3H/HEJ mice 25 days after infection with C3b or (C) uninfected C3H/HEJ mice reacted with 34-1-2S. (D) ganglionic neurons from C3H/HEJ mice 25 days after infection with S-130 did not stain with ID4.5. Cell smears were lightly counterstained with rapid haematoxylin. Magnification is x 384 (A-D).


A
B
C
D

4.6 Effect of H-2K^d expression in mice of a different H-2 haplotype on persistence of reactivatable virus

The ability of S-130 and C3b to persist in mouse sensory ganglia was assessed using a classical explant reactivation technique.

Groups of ten C57BL10 mice were infected (day 0) with S-130 or C3b and on day 32 spinal ganglia were removed for explant culture. Virus could be reactivated from 6/10 S-130 and 5/9 C3b infected mice. It was concluded that S-130 and C3b reactivate with comparable efficiency ie. expression of H-2K^d in H-2^b neurons during the productive phase of infection had no measurable effect on latency, as assessed by reactivation *in vitro*. It is not known whether H-2K^d was expressed in the neurons from which virus was reactivated.

4.7 Effect of H-2K^d expression in mice of a different H-2 haplotype on the rate of virus clearance from skin of mice adoptively transferred with H-2^d restricted anti-HSV immune cells

Following ear inoculation of mice, it has been shown that virus titres at the site of inoculation peak 3 to 5 days after infection and decline rapidly over the next 3 days (Nash *et al.,* 1980 b). The experiments described here were designed to determine if adoptive transfer of immune cells enhanced clearance of virus.

To gain familiarity with adoptive transfer procedures, draining lymph node (DLN) cells obtained from SC16 infected BALB/c (H-2^d) or CBA (H-2^k) mice seven days after inoculation were adoptively transferred into groups of 15 CBA recipient mice. An additional group of 15 CBA mice were injected with PBS rather than DLN cells. Mice were infected in the pinna of the left ear (day 0) with SC16 immediately after receiving DLN cells or PBS. Infectious virus was quantified in ear homogenates from groups of 5 mice killed 1, 3 and 5 days after infection (Figure 4.11).

Adoptive transfer of CBA DLN cells reduced virus titres in the skin of CBA mice by 0.7 and 1.0 logs on days 3 and 5 respectively, compared with animals that did not receive DLN cells. As expected, transfer of DLN cells from HSV infected BALB/c mice had no effect on virus titres in CBA mice.

To determine whether H-2^d restricted anti-HSV immune cells accelerated clearance of virus from mice of a different haplotype expressing S-130 encoded H-$2K^d$ antigens, DLN cells from SC16 infected BALB/c (H-2^d) or CBA (H-2^k) mice were transferred into CBA mice that were immediately infected (day 0) with S-130 (10 mice) or C3b (10 mice). As controls, CBA mice were injected with PBS rather than DLN cells and infected with S-130 (10 mice) or C3b (10 mice). Infectious virus was quantified in ear homogenates from groups of 5 mice killed on day 1 and day 3 after infection (Figure 4.12).

Figure 4.11

Titres of infectious HSV-1 in ears of CBA mice infected with SC16 following adoptive transfer of immune DLN cells from SC16 infected BALB/c mice (open squares) or CBA mice (closed circles). Control mice received no DLN cells (closed triangles). Virus yield was expressed as $\log_{10}$ pfu. Each point represents the geometric mean titre and range from groups of 5 mice.

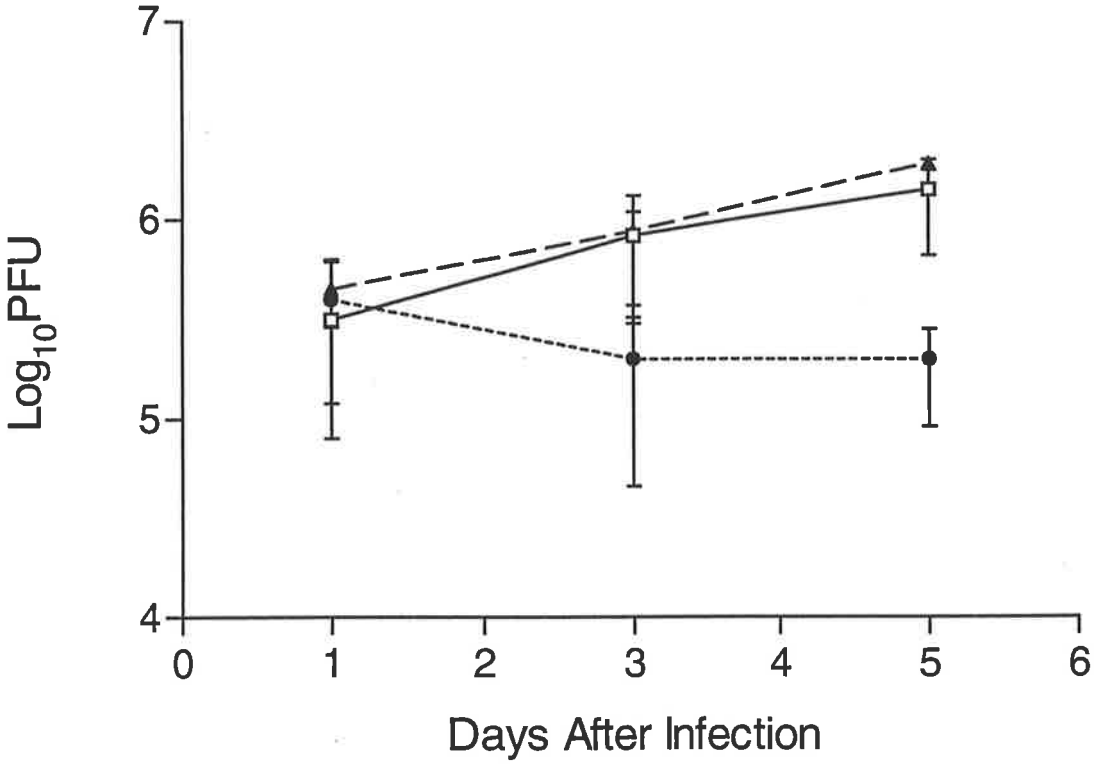

7
6
5
4
$Log_{10}PFU$
0
1
2
3
4
5
6
Days After Infection

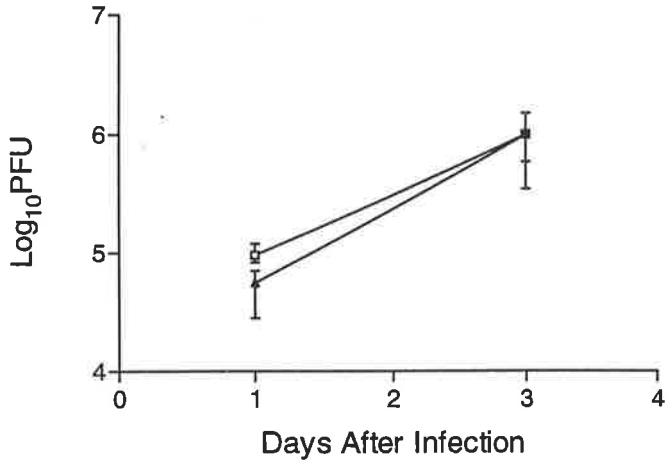
Figure 4.12

Titres of infectious HSV-1 in ears of CBA mice infected with S-130 (open squares) or C3b (closed triangles) following adoptive transfer of (A) no DLN cells, (B) immune DLN cells from SC16 infected BALB/c mice and (C) immune DLN cells from SC16 infected CBA mice. Virus yield was expressed as $\log_{10}$ pfu. Each point represents the geometric mean titre and range from groups of 5 mice.

A.


7
6
5
4
$Log_{10}PFU$
0
1
2
3
4
Days After Infection

B.


7
6
5
4
$Log_{10}PFU$
0
1
2
3
4
Days After Infection

C.


7
6
5
4
$Log_{10}PFU$
0
1
2
3
4
Days After Infection

Compared with control animals, CBA DLN cells reduced virus titres in the ears of C3b and S-130 infected mice by approximately 0.9 and 0.8 logs, respectively, on day 3. In contrast, BALB/c DLN cells had no effect. It was concluded that DLN cells from SC16 infected BALB/c mice had no detectable effect on virus titres in the skin of S-130 infected recipient CBA mice. The implications and limitations of this experiment are discussed in Chapter 6.

4.8 Summary

S-130 was shown by immunohistochemistry and *in situ* hybridization to infect primary sensory neurons following cutaneous inoculation of mice. Although H-2K^d RNA transcripts could not be localized to neurons by ISH, they were detected by northern blot analysis of RNA extracted from ganglia productively infected with S-130. S-130 infected primary sensory neurons were shown by immunohistochemistry to synthesize H-2K^d antigens. Further, rosetting studies demonstrated that H-2K^d antigens were expressed at the surfaces of S-130 infected neurons. There was no significant difference between S-130 and C3b infected H-2K^b mice with respect to rates of clearance of infectious virus from skin and ganglia and viral DNA from ganglia. H-2K^d antigens could be detected in neurons latently infected with S-130 and reactivation studies showed that expression of H-2K^d in neurons of H-2^b mice had no measurable effect on latency.

The experiments presented in this chapter demonstrated that HSV infected neurons can express cell-surface H-2K^d molecules. However, the limited functional studies done were unable to show any effect of this H-2K^d expression *in vivo*.

5. ANALYSIS OF β_2M, TAP-1, TAP-2 AND ICAM-1 GENE EXPRESSION IN SPINAL GANGLIA OF HSV INFECTED MICE

In addition to αC synthesis, stable, functional MHC-Ia expression on the surfaces of cells requires the expression of β_2m and TAP gene products. It has been shown that β_2m transcription (Pereira *et al.*, 1994) and cell-surface expression (R. Pereira, personal communication) is upregulated in the peripheral nervous systems of HSV (strain SC16) infected mice. Having established that H-2K^d is expressed on the surfaces of S-130 infected neurons (Chapter 4), it was reasoned that not only β_2m but also TAP gene products must be upregulated in S-130 infected spinal ganglia. Therefore, the effect of S-130 infection on the synthesis of these molecules in spinal ganglia was examined.

The effect of HSV infection on ICAM-1 expression was also studied because ICAM-1 is an adhesion molecule involved in stabilization of cell to cell contact during CTL mediated lysis.

5.1 β_2m, TAP-1 and TAP-2 RNA synthesis

To address the hypothesis that transcription of β_2m, TAP-1 and TAP-2 mRNA is upregulated in HSV infected spinal ganglia, RNA samples from infected and uninfected mice were compared by northern blot hybridization using probes specific for the relevant molecules.

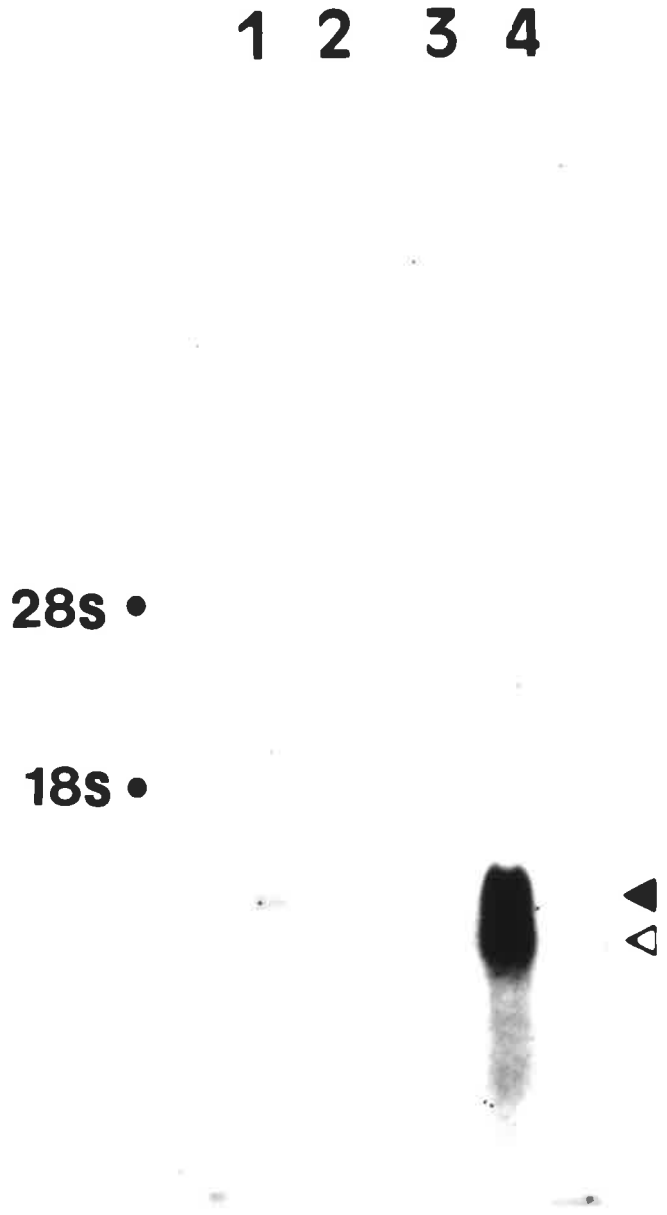
Ten adult C3H/HEJ mice were infected (day 0) with S-130. On day 5, RNA samples (5μg) from spinal ganglia were tested by northern blot hybridization for the presence of β_2m, TAP-1 and TAP-2 RNA transcripts using probes generated from pGem3β_2(A), mtp-1 and mtp-2, respectively. RNA extracted from uninfected mice and Vero cells were tested concomitantly. RNA extracted from EL4 cells, which constitutively express H-2K^b antigens was included as a positive control for the presence of β_2m, TAP-1 and TAP-2 mRNAs.

The probe generated from pGem3β_2(A) detected two RNA species, corresponding to the expected migration of mouse β_2m RNA transcripts (Morello *et al.*, 1982) (Figure 5.1). Probe did not hybridize to RNA extracted from Vero cells or uninfected mouse ganglia.

The nitrocellulose filter was stripped of bound β_2m probe and was hybridized with a probe generated from mtp-1. A duplicate filter (which had not been previously probed) was hybridized with a probe generated from mtp-2. In

Figure 5.1

Detection of β_2-microglobulin RNA by northern blot hybridization and phosphor imaging (16 hour exposure). RNA samples (5μg) were electrophoresed, transferred to nitrocellulose and hybridized to a ^{32}P-labelled DNA probe derived from pGem3β_2(A). Lanes 1 and 2 contain RNA from EL4 and Vero cells, respectively. Lane 3 contains RNA from uninfected C3H/HEJ mouse spinal ganglia and lane 4 contains RNA from spinal ganglia of C3H/HEJ mice productively infected with S-130. 1.0kb and 0.8kb β_2-microglobulin mRNAs are indicated as closed and open arrowheads, respectively. Positions of the 1.9kb 18s and 4.8kb 28s ribosomal RNAs are indicated.


1 2 3 4
28s •
18s •

RNA extracted from S-130 infected ganglia, mtp-1 and mtp-2 probes detected TAP-1 (Figure 5.2 A) and TAP-2 (Figure 5.3 A) RNAs, respectively.

To determine whether the same amount of RNA had been loaded into each track, both filters were reprobed for transcripts of a cellular housekeeping gene mouse β-actin (Alonso *et al.,* 1986) (Figure 5.2 B & 5.3 B). Compared with the sample from uninfected ganglia, gels had been overloaded with three times more RNA from infected ganglia and 1.5 times more RNA from Vero cells. However, taking into account the loading differences, it was still possible to conclude that β_2m, TAP-1 and TAP-2 transcription was upregulated in S-130 infected ganglia.

5.2 β_2m and TAP-2 antigen expression

Expression of cell-surface β_2m by neurons was determined by rosetting and TAP-2 antigen expression was examined immunohistochemically in HSV infected ganglia.

5.2.1 Detection of cell-surface β_2m by rosetting

Ten adult C57BL10 mice were infected (day 0) with S-130. On day 5, spinal ganglia were removed and cell suspensions prepared. Cell suspensions were tested for cell-surface β_2m molecules using an anti-C57BL10 (H-2K^b) β_2m monoclonal antibody (S19.8) and prot A-SRBC in a rosetting assay. The use of

Figure 5.2

(A) Detection of TAP-1 RNA by northern blot hybridization and phosphor imaging (16 hour exposure). RNA samples (5μg) were electrophoresed, transferred to nitrocellulose and hybridized with a ^{32}P-labelled DNA probe derived from mtp-1. Lanes 1 and 2 contain RNA from EL4 and Vero cells, respectively. Lane 3 contains RNA from uninfected C3H/HEJ mouse spinal ganglia and lane 4 contains RNA from spinal ganglia of C3H/HEJ mice productively infected with S-130. The 2.6kb TAP-1 RNA species is arrowed. Positions of the 1.9kb 18s and 4.8kb 28s ribosomal RNAs are indicated.

(B) Reprobe of (A) with a ^{32}P-labelled DNA probe derived from pAL41 (16 hour exposure), showing the distribution of cellular β-actin transcripts in all RNA samples loaded (lanes 1-4).

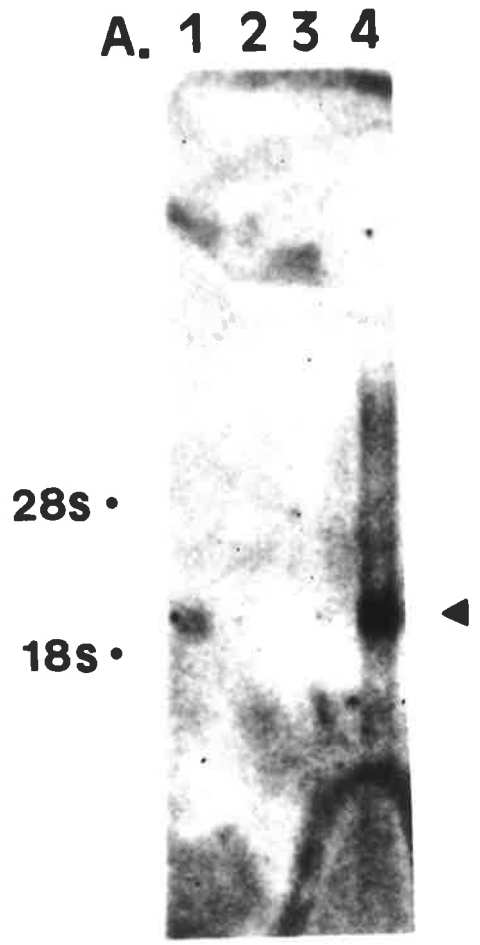
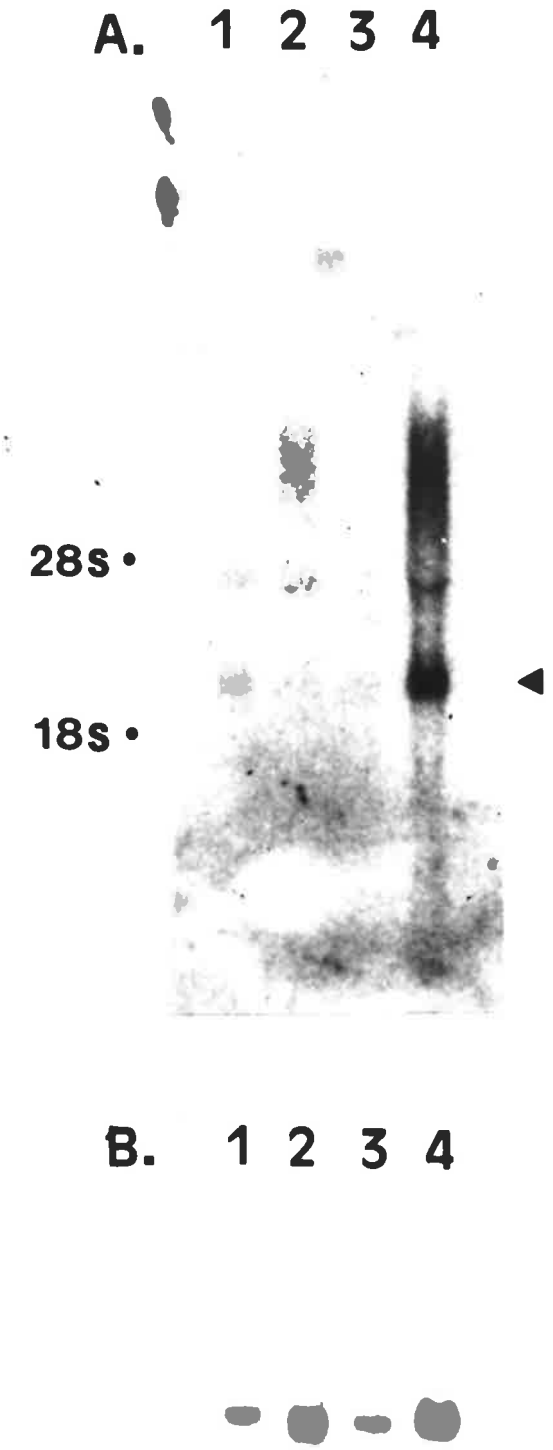

A. 1 2 3 4
28s •
18s •
B. 1 2 3 4

Figure 5.3

(A) Detection of TAP-2 RNA by northern blot hybridization and phoshor imaging (16 hour exposure). RNA samples (5μg) were electrophoresed and transferred to nitrocellulose and hybridized with a ^{32}P-labelled DNA probe derived from mtp-2. Lanes 1 and 2 contain RNA from EL4 and Vero cells, respectively. Lane 3 contains RNA from uninfected C3H/HEJ mouse spinal ganglia and lane 4 contains RNA from spinal ganglia of C3H/HEJ mice productively infected with S-130. The 2.4kb TAP-2 RNA species is arrowed. Positions of the 1.9kb 18s and 4.8kb 28s ribosomal RNAs are indicated.

(B) Reprobe of (A) with a ^{32}P-labelled DNA probe derived from pAL41 (16 hour exposure), showing the distribution of cellular β-actin transcripts in all RNA samples loaded (lanes 1-4).


A. 1 2 3 4
28s •
18s •
B. 1 2 3 4

this antibody necessitated the use of C57BL10 mice. EL4 (H-2^b) cells were included as a control for the presence of surface β_2m. Cell preparations from uninfected ganglia were included to determine whether uninfected neurons express cell-surface β_2m. Monoclonal antibody, ID4.5, was used to determine the extent of non-specific rosetting.

Seven percent of neurons derived from ganglia productively infected with S-130 (eg. Figure 5.4 A) and 1.5% of neurons from uninfected ganglia (eg. Figure 5.4 B) formed rosettes with S19.8 (Table 5.1). Neurons from S-130 infected ganglia did not form any rosettes with ID4.5 (Figure 5.4 C). It was concluded that there is an upregulation of surface β_2m expression on ganglionic neurons *in vivo* in response to HSV infection. By day 13, up to 60% of neurons in HSV infected ganglia have been found to be cell-surface β_2m positive (R. Pereira, personal communication).

5.2.2 Immunohistochemical detection of TAP-2 antigens in cell lines

TAP-2 expression was tested in mouse MHC-Ia positive (P815) and MHC-Ia deficient (Neuro-2a) cell lines. To control for specificity of staining, monkey and human cells (Vero and IMR-32, respectively) were reacted with the antiserum, which is directed against recombinant mouse TAP-2. In addition, all cell smears were reacted with NRS.

Figure 5.4

Photomicrographs showing detection of cell-surface β_2-microglobulin by rosetting. (A) S19.8 reacted with dissociated neurons in ganglionic cell preparations from C57BL10 mice productively infected with S-130. (B) a view of dissociated mouse ganglionic neurons from uninfected C3H/HEJ mice showing rosetted (arrow) and non-rosetted (arrowhead) neurons. (C) typical view of dissociated ganglionic neurons from C3H/HEJ mice productively infected with S-130 reacted with ID4.5. Cells were lightly counterstained with methyl violet. Magnification is x 300 (A) and x 200 (B & C).

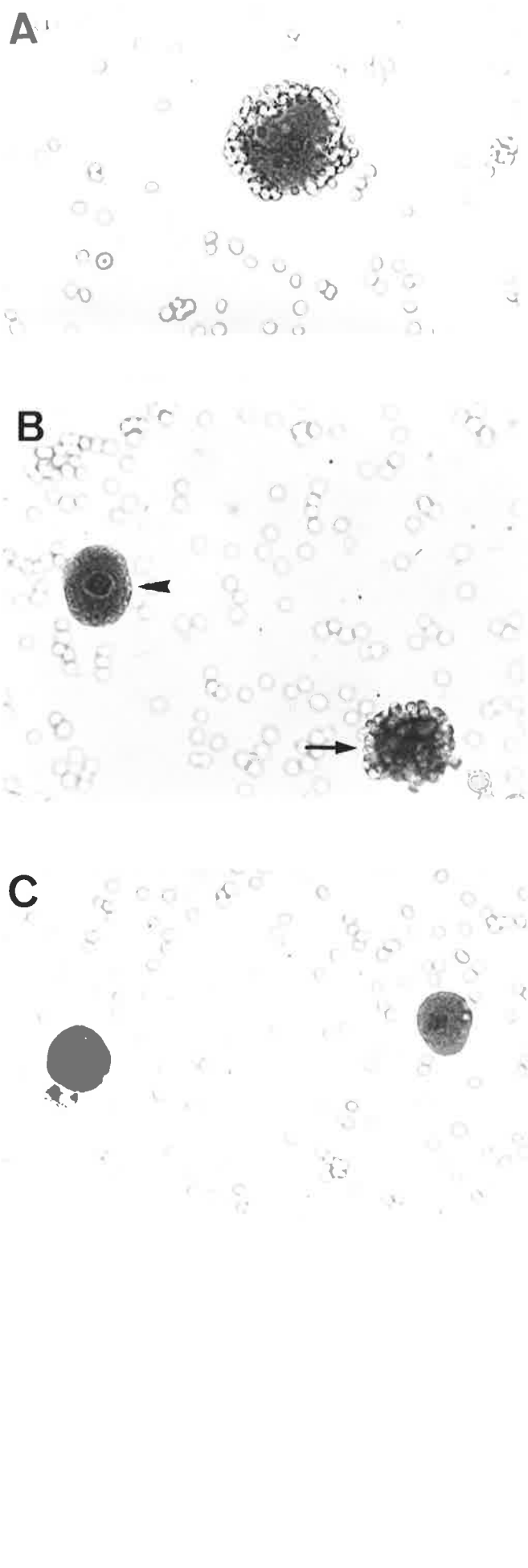

A
B
C

Table 5.1 Detection of Surface β_2m Antigens on Primary Sensory Neurons from HSV Infected C57BL10 Mice

Cell Type	Antibody	Number RFC*/Total Counted (%)	% Viral Antigen Positive Neurons
Neurons From HSV Infected Ganglia (Day 5 P.I.)	S19.8 ID4.5	94/1350 (7.0) 0/1350 (0)	20
Uninfected Neurons	S19.8 ID4.5	20/1350 (1.5) 0/1350 (0)	0
Uninfected EL4	S19.8 ID4.5	926/945 (98.0) 0/945 (0)	N/A

* Rosette forming cells
N/A Not applicable

TAP-2 specific perinuclear staining was reproducibly detected in P815 cells (Figure 5.5 A). No staining was observed in Neuro-2a, Vero or IMR-32 cells (Figure 5.5 B, C & D, respectively). P815 cells reacted with NRS were not stained (Figure 5.5 E). It was concluded that the rabbit anti-mouse TAP-2 antiserum specifically detected mouse TAP-2 proteins. Contrary to expectation, only a minority (~10%) of P815 cells bound anti-mouse TAP-2 antiserum. However, to date, this antiserum raised against recombinant mouse TAP-2 has been used only in immunoprecipitation studies (Suh *et al.*, 1994) and its efficacy in immunohistochemical reactions is unknown. Other antibodies to mouse TAP-2 were not available.

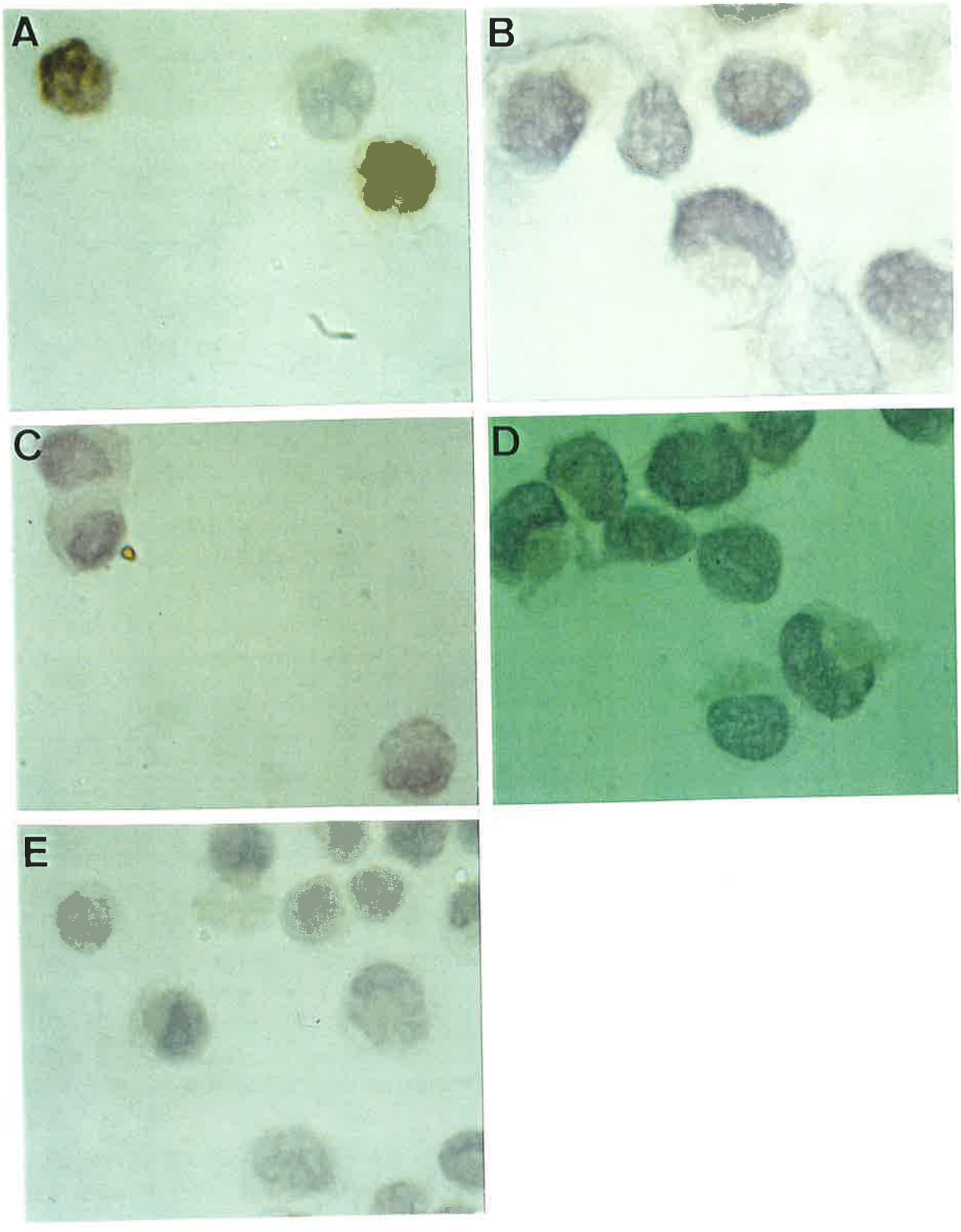
5.2.3 Immunohistochemical detection of TAP-2 antigens in HSV infected spinal ganglia

To determine whether TAP-2 antigens are expressed in HSV infected dorsal root ganglia, paraffin sections of PLP-fixed tissue and dissociated ganglionic cell smears were examined immunohistochemically using rabbit anti-mouse TAP-2 antiserum.

Twenty adult C3H/HEJ mice were infected (day 0) with S-130. On day 5, spinal ganglia from 10 mice were removed, PLP fixed and paraffin embedded. The remaining 10 mice were used to prepare cell smears from pooled ganglia. P815 cell smears were included as a positive control. Paraffin sections and cell smears from uninfected ganglia and NRS were included to control for the

Figure 5.5

Photomicrographs showing detection of TAP-2 proteins by immunohistochemistry (brown staining). (A) specific staining with rabbit anti-mouse TAP-2 antiserum was detected in P815 cells. Specific staining was not detected in (B) Neuro-2a, (C) Vero and (D) IMR-32 cells reacted with rabbit anti-mouse TAP-2 antiserum or (E) P815 cells reacted with normal rabbit serum. Cell smears were lightly counterstained with rapid haematoxylin. Magnification is x 960 (A-E).


A
B
C
D
E

specificity of staining.

Attempts to detect binding of antiserum to 5μm sections of PLP-fixed tissue and dissociated ganglionic cell smears were unsuccessful, despite detection of TAP-2 in P815 cells. It was reasoned that fixation or processing of ganglionic tissue or cells may have affected the integrity of TAP-2 antigens. Labile proteins are optimally preserved by freezing, and therefore further attempts to detect TAP-2 were made in frozen ganglionic sections.

Ten adult C3H/HEJ mice were infected (day 0) with S-130. On day 5 spinal ganglia were pooled, frozen and 6μm sections were tested immunohistochemically for TAP-2. P815 cells were included as a positive control. Uninfected mouse ganglionic frozen sections and NRS were included as controls for specificity of staining. TAP-2 was detected in P815 cells but not in HSV infected or uninfected spinal ganglia (not illustrated). No staining was seen in P815 cells or ganglionic tissue reacted with NRS.

Pereira *et al.* (1994) showed that β_2m expression in ganglia increases from 5 to 8 days after HSV infection. By extrapolation it was reasoned that TAP-2 expression in infected ganglia may increase with time. Therefore, a further attempt to detect TAP-2 was made using spinal ganglia removed not only 5 but also 8 days after infection with S-130 (20 mice).

Eight days after infection with S-130 (Figure 5.6 A & B) TAP-2 was detected in satellite and Schwann cells, but staining of neurons was equivocal. No staining was detected in uninfected ganglionic tissue (Figure 5.6 C & D) or in S-130 infected ganglionic sections reacted with NRS (Figure 5.6 E). Despite several attempts TAP-2 could not be detected in spinal ganglia removed 5 days after infection with S-130, suggesting that the level of TAP-2 expression was below the limit of detection.

5.3 ICAM-1 expression

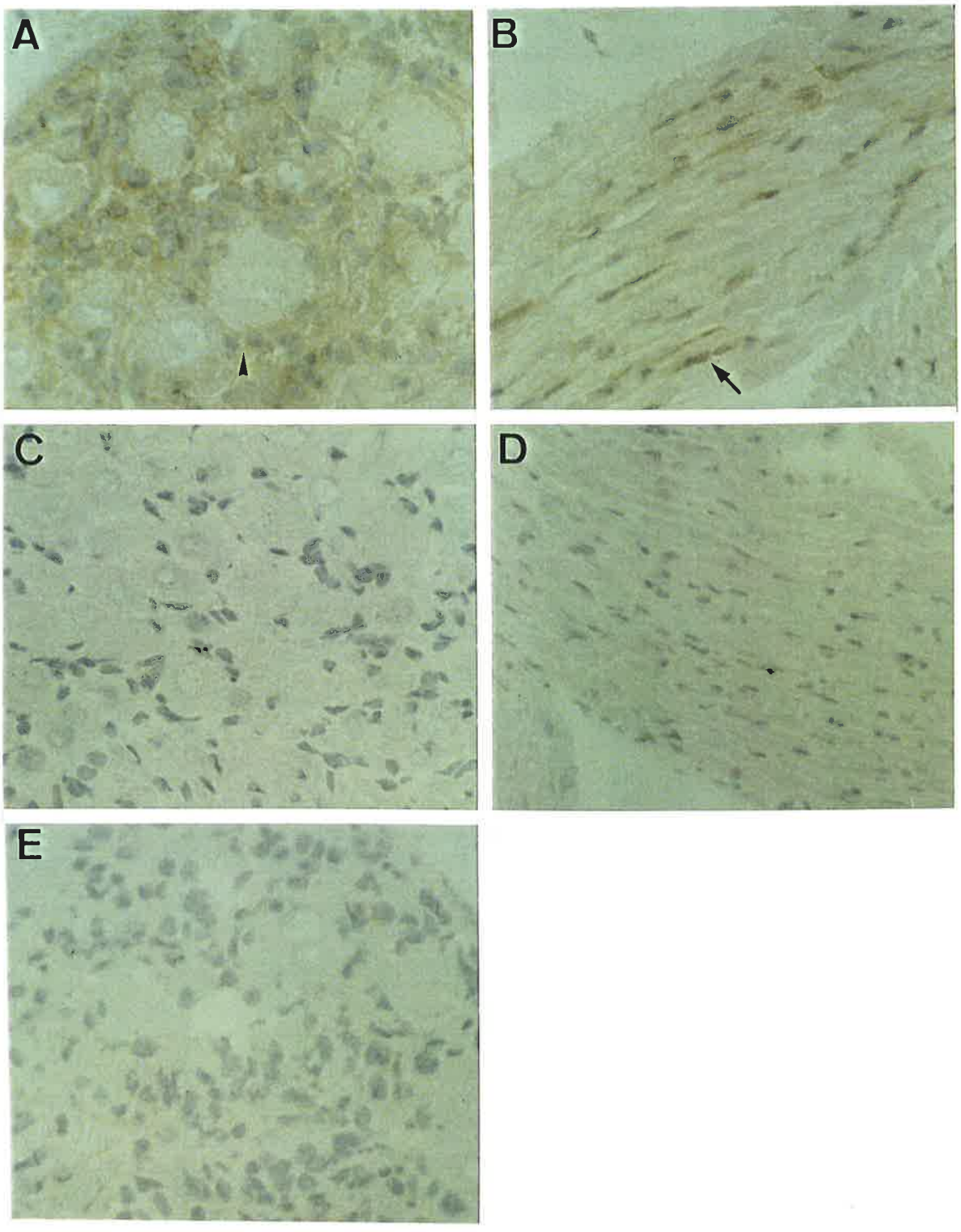
To address the hypothesis that ICAM-1 expression is upregulated in HSV infected spinal ganglia, ganglionic RNA samples and cell preparations from infected and uninfected mice were compared by northern blot hybridization and rosetting, respectively.

5.3.1 Northern blot hybridization

Ten C3H/HEJ mice were infected (day 0) with S-130. Ganglionic RNA samples (5μg) prepared on day 5 were analysed with a probe generated from EH3KpBST, which contains mouse ICAM-1 cDNA. RNA from uninfected ganglia and Vero cells were tested concomitantly. RNA extracted from NS1 cells, which constitutively express ICAM-1, was included as a control for the presence of ICAM-1 mRNA.

Figure 5.6

Photomicrographs showing detection of TAP-2 proteins by immunohistochemistry (brown staining). (A & B) rabbit anti-mouse TAP-2 antiserum reacted with frozen ganglionic sections from C3H/HEJ mice 8 days after infection with S-130. Arrowhead shows satellite cell staining and arrow shows nerve fibre staining in Schwann cells. Note the lack of detectable TAP-2 specific staining in neurons. Specific staining was not detected in (C) ganglia or (D) nerve from uninfected C3H/HEJ mice reacted with rabbit anti-mouse TAP-2 antiserum or (E) in ganglionic sections from C3H/HEJ mice 8 days after S-130 infection reacted with normal rabbit serum. Ganglionic sections were lightly counterstained with rapid haematoxylin. Photomicrograph magnification is x 300 (A, B, D & E) and x 200 (C).


A
B
C
D
E

ICAM-1 mRNA was detected in S-130 infected ganglia and NS1 cells (Figure 5.7 A). Probe did not hybridize to RNA extracted from Vero cells or uninfected ganglia. Reprobing the filter for mouse β-actin, verified that loading of all RNA samples was comparable (Figure 5.7 B). It was concluded that ICAM-1 mRNA transcription is upregulated in HSV infected sensory ganglia.

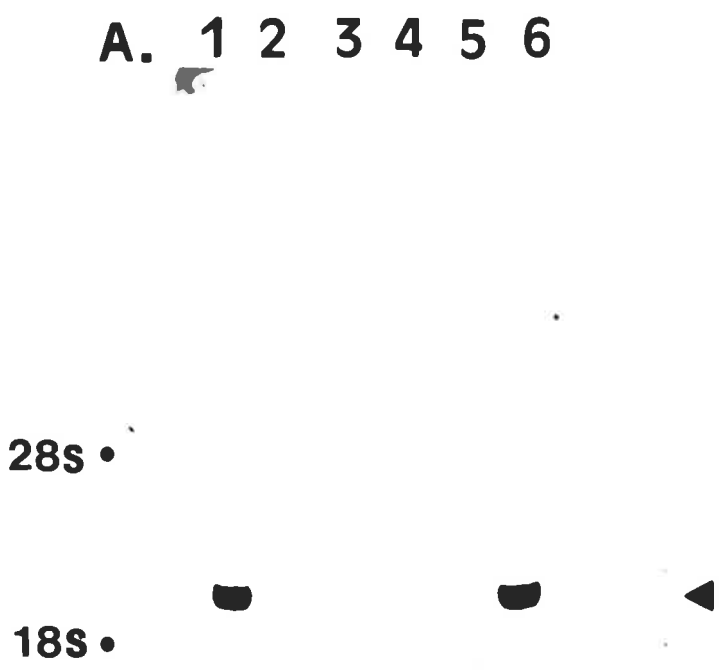
5.3.2 Detection of cell-surface ICAM-1 by rosetting

Ten adult C3H/HEJ mice were infected (day 0) with S-130. On day 8, cell suspensions prepared from pooled spinal ganglia were tested for surface ICAM-1 expression using an anti-mouse ICAM-1 monoclonal antibody (YN1/1.7.4) and prot A-SRBC in a rosetting procedure. Day 8 was chosen for this study because in previous experiments, ICAM-1-specific rosettes could not be detected on neurons from HSV infected ganglia on day 5, although they were detected on a proportion (9.0%) of non-neuronal ganglionic cells. Capped splenocytes from uninfected C3H/HEJ mice were included as a positive control for the presence of surface ICAM-1 (Takei *et al.*, 1985). Cell preparations from uninfected ganglia were included to determine whether uninfected neurons express cell-surface ICAM-1. A monoclonal antibody (M1/9.3.4.HL.2) against a lymphocyte marker, CD45 was included to (i) establish the percentage of lymphocytes in ganglionic cell preparations and (ii) determine the extent of non-specific rosetting on neurons.

Figure 5.7

(A) Detection of ICAM-1 RNA by northern blot hybridization and phosphor imaging (24 hour exposure). RNA samples (5μg) were electrophoresed, transferred to nitrocellulose and hybridized with a ^{32}P-labelled DNA probe derived from EH3KpBST. Lanes 1 and 3 contain RNA from NS1 and Vero cells, respectively. Lane 4 contains RNA from uninfected C3H/HEJ mouse spinal ganglia and lane 6 contains RNA from spinal ganglia of C3H/HEJ mice productively infected with S-130. A 2.5kb ICAM-1 RNA species is arrowed. Positions of the 1.9kb 18s and 4.8kb 28s ribosomal RNAs are indicated.

(B) Reprobe of (A) with a ^{32}P-labelled DNA probe derived from pAL41 (16 hour exposure), showing the distribution of cellular β-actin transcripts in all RNA samples loaded (lanes 1, 3, 4 and 6).


A. 1 2 3 4 5 6
28s •
18s •


B. 1 2 3 4 5 6

Rosette forming primary sensory neurons were readily identified by their large mean diameter (~20-70μm; mean 30μm) and characteristic cell morphology. All cells counted as non-neuronal small cells were <10μm in diameter and were readily distinguishable from neurons (Figure 5.8, Table 5.2).

Typically <7% of small cells from uninfected or S-130 infected mouse ganglia formed rosettes when reacted with M1/9.3.4.HL.2, suggesting that the majority of small cells were not lymphocytes and likely to be satellite cells and nerve Schwann cells. In preparations from S-130 infected ganglia reacted with YN1/1.7.4., small cells readily formed rosettes on day 8 (Table 5.2). In contrast, small cells from uninfected ganglionic preparations reacted with YN1/1.7.4 rarely formed rosettes. Eight days after infection, a very small proportion of neurons from S-130 infected ganglia formed rosettes with YN1/1.7.4. Neurons from S-130 infected or uninfected ganglia did not form rosettes with M1/9.3.4.HL.2. It was concluded that a significant proportion of small ganglionic cells (ie glial cells) but only a small minority of neurons derived from ganglia 8 days after infection with S-130 expressed detectable levels of cell-surface ICAM-1.

Figure 5.8

Photomicrographs showing detection of cell-surface ICAM-1 expression by rosetting. YN1/1.7.4 was reacted with dissociated ganglionic cells from C3H/HEJ mice 8 days after infection with S-130. (A) typical view of a rosetted primary sensory neuron and (B; arrow) small cell. For comparative purposes a non-rosetted neuron is indicated (arrowhead). (C) higher power of a rosetted small cell is shown. Typical views of a non-rosetted neurons from (D) uninfected C3H/HEJ mice reacted with YN1/1.7.4. and (E) S-130 infected C3H/HEJ mice (8 days after infection) reacted with M1/9.3.4.HL.2. Cells were counterstained with methyl violet. Magnification is x 750 (A & C) and x 200 (B, D & E).

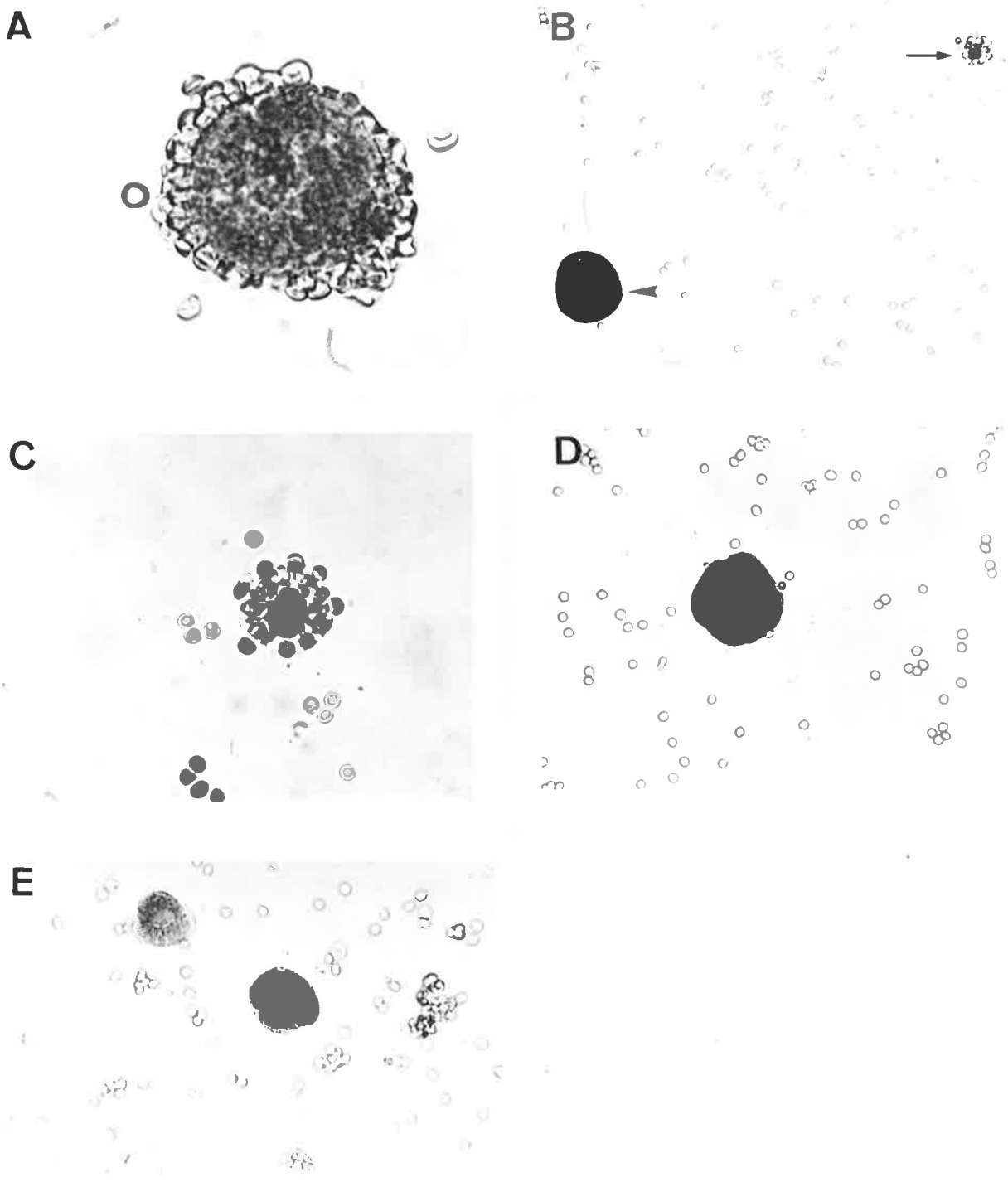

A
B
C
D
E

Table 5.2 Detection of Surface ICAM-1 Antigens on Ganglionic Cells After HSV Infection of C3H/HEJ Mice

Treatment	Cell Type	Antibody	No. RFC*/Total Counted (%).
Day 8 HSV Infection	Neurons	YN1/1.7.4	20/790 (2.5)
		M1/9.3.4.HL.2	0/700 (0)
	Small Cells[a]	YN1/1.7.4	61/360 (17)
		M1/9.3.4.HL.2	20/323 (6.2)
Uninfected	Neurons	YN1/1.7.4	0/490 (0)
		M1/9.3.4.HL.2	0/300 (0)
	Small Cells[a]	YN1/1.7.4	10/600 (1.7)
		M1/9.3.4.HL.2	10/507 (2.0)
Uninfected	Spleen Cells	YN1/1.7.4	381/810 (47)
		M1/9.3.4.HL.2	315/731 (43)

[a] Small Cells represents non-neuronal ganglionic cells.

* Rosette forming cells

6. DISCUSSION

Experiments presented in this thesis showed that H-2K^d antigens can be synthesized and transported to the surfaces of HSV infected neurons following introduction, by S-130, of an exogenous H-2K^d cDNA, under independent regulatory control. Cell-surface expression of uncomplexed αC alone is, at best, at very low levels and has not been reported for H-2K^d (Allen *et al.,* 1986; Hansen *et al.,* 1988; Oritz-Navarrete & Hammerling, 1991; Bix & Raulet, 1992; Neefjes *et al.,* 1992) and therefore it is highly likely that detection of H-2K^d in the cytoplasms and on the surfaces of S-130 infected cells indicates the formation of stable ternary complexes. This proposal is supported by the studies of Joly *et al.* (1991) and Rall *et al.* (1995) who showed that delivery and expression of αC alone in cells of neuronal origin and neurons *in vivo*, was sufficient to obtain functional, cell-surface MHC-Ia expression. Further, in the absence of TAPs and β_2m, αCs are retained in the ER by calnexin, rather than being transported to the cell-surface (Degen *et al.,* 1992; Jackson *et al.,* 1994; Rajagopalan *et al.,* 1994) and degradation of αCs is rapid unless they are complexed with β_2m and peptides.

In this study, no evidence was found that HSV infection interferes with cell-surface MHC-Ia expression in mouse neurons. Interestingly, a HSV IE protein, ICP47, can interfere with TAP function in fibroblasts, preventing the formation of stable ternary complexes (York *et al.,* 1994; Hill *et al.,* 1994; Hill *et al.,*

1995; Fruh *et al.,* 1995). This effect appears to be host and cell type specific, in that it has only been demonstrated in fibroblast and epitheliod cells of human origin and not in mouse cells (Fruh *et al.,* 1995; Hill *et al.,* 1995). Despite strong sequence and protein homology between human and murine TAPs (Monaco, 1992; Townsend & Trowsdale, 1993), introduction of murine TAP-1 and TAP-2 into ICP47-expressing human (HeLa) cells does not inhibit cell-surface MHC-Ia expression (Fruh *et al.,* 1995). Furthermore, ICP47 does not appear to prevent antigen presentation in HSV infected human B cells because they can be lysed by HSV-specifc $CD8^+$ CTLs (Posavad & Rosenthal, 1992; Koelle *et al.,* 1993). In the present study, cell-surface H-$2K^d$ antigens were readily detected on human IMR-32 and K562 cells infected with S-130. In the case of K562 cells, H-$2K^d$ was shown to be associated with β_2m.

Attempts were made to show directly that TAPs are upregulated in neurons in HSV infected ganglia. TAP-1 and TAP-2 transcription was shown by northern blotting to be upregulated in ganglia productively infected with HSV (see section 5.1) and TAP-2 antigens could not be localized to neurons by immunohistochemistry. However, TAP-2 was detected in satellite glial cells and peripheral nerve Schwann cells, 8 days after infection. TAP-2 is perinuclear (Kleijmeer *et al.,* 1992) and in neurons, which are very large, TAP-2 may have been below the threshold of detection. In this respect, it has been reported previously that TAP proteins are expressed in low amounts, making immunohistochemical detection, even in cultured cells, problematic

(Hill *et al.,* 1995). Although ICP47 may interfere with TAP function, there is no evidence that HSV infection affects TAP expression. This is in contrast to the highly oncogenic adenovirus, Ad12 (Rotem-Yehudar *et al.*, 1994).

Several viruses, other than HSV, have been shown to downmodulate MHC-Ia expression by a variety of mechanisms:

(i) HCMV downregulates cell-surface MHC-Ia expression (Browne *et al.,* 1990; Barnes & Grundy, 1992; Waldman *et al.,* 1992; Gilbert *et al.,* 1993; Yamashita *et al.,* 1993) at a post-translational level (Beersma *et al.,* 1993; Warren *et al.,* 1994; Yamashita *et al.,* 1994; Jones *et al.,* 1995).

(ii) MCMV early gene product has the ability to downregulate MHC-Ia expression (Del Val *et al.,* 1989; Campbell *et al.,* 1992; Campbell & Slater, 1994) by impeding the transport of MHC-Ia-peptide complexes from the ER to golgi (Del Val *et al.,* 1992; Thale *et al.,* 1995). This pattern of interference by MCMV on MHC-Ia complex formation is similiar to that caused by subgroup B-E adenoviruses (Kvist *et al.,* 1978; Burgert & Kvist, 1985; Andersson *et al.,* 1985; Paabo *et al.,* 1986; Burgert *et al.,* 1987; Wold & Gooding, 1991; Hermiston *et al.,* 1993) in which the E3-gp 19KDa glycoprotein binds to MHC-Ia molecules and retains them in the ER (Andersson *et al.,* 1987; Paabo *et al.,* 1987; Jefferies & Burgert, 1990).

(iii) EBV selectively downregulates expression of certain MHC-Ia alleles in Burkitt's lymphoma cells (Masucci *et al.,* 1987; Masucci *et al.,* 1989; Andersson *et al.,* 1991; Masucci *et al.,* 1992).

(iv) Mouse hepatitis virus also appears to selectively modulate specific MHC-Ia alleles (Joseph *et al.,* 1989).

(v) PRV has been shown to interfere with MHC-Ia expression but, at the present time, the mechanism of this interference is unknown (Mellencamp *et al.,* 1991).

Given that β_2m is required for cell-surface expression of stable MHC-Ia ternary complexes, is there evidence that expression of this molecule is upregulated in the nervous system in response to HSV? Pereira *et al.* (1994) showed previously that β_2m mRNA transcripts accumulate transiently in sensory neurons and glial cells in ganglia of HSV infected mice; this was confirmed in the present study. Further, β_2m was detected on the surfaces of a minority of neurons in HSV infected ganglia 5 days after flank inoculation (this study) and a high proportion (60%) 13 days after infection (R. Pereira, personal communication). It has not been shown formally that β_2m is upregulated in neurons infected with HSV, as opposed to neighbouring uninfected neurons. However, the finding that S-130 infected K562 cells express cell-surface β2m in association with H-2K^d suggests that there is no fundamental problem with

β_2m expression in HSV infected cells. Further, HSV infection obviously does not prevent endogenously synthesized β_2m from complexing with αCs.

The cytokines responsible for upregulation of MHC-Ia molecules, including β_2m and TAPs, are not known. *In vitro*, some cells of neuronal origin respond to γ-IFN (Lampson & Fisher, 1984; Main *et al.,* 1988; Joly & Oldstone *et al.,* 1992), whereas others do not (Drew *et al.,* 1993). It is therefore possible that other, perhaps uncharacterized cytokines are responsible for stimulating the synthesis of MHC-I molecules *in vivo.*

A property of cell-surface MHC-Ia antigens is that they are potent alloantigens, which can be recognized by alloreactive CTLs. However, recognition by allogeneic T-cells, of H-2K^d antigens expressed on the surfaces of S-130 infected Vero and L929 cells was not detected in this study. There are several potential explanations for this result:

(i) H-2K^d may have been expressed on the surfaces of S-130 infected cells as uncomplexed αCs. However, this is considered unlikely for the reasons already discussed.

(ii) As few as 200 MHC-Ia-peptide complexes are required for CTL recognition and lysis (Christinck *et al.,* 1991). Therefore, the level of cell-surface H-2K^d antigens expressed may have been below the threshold recognized by anti-H-2K^d CTLs.

(iii) The experimental design for studying alloreactive CTLs may have been flawed. It is possible that alloreactive CTLs raised against P815 cells cannot recognize H-2K^d expressed in Vero or L929 cells, particularly if peptides derived from the cytoplasm are involved in allorecognition.

(iv) Co-synthesized viral and MHC-Ia antigens may associate immediately, reducing the availability of self-peptides for recognition by alloreactive CTLs. Coupar *et al.* (1986) raised this possibility after finding that H-2K^d antigens expressed by a H-2K^d VV recombinant are, at best, weak alloantigens.

Several groups have demonstrated MHC-Ia restricted CTL responses against virally infected cultured cells, expressing exogenous MHC-Ia αCs (Coupar *et al.,* 1986; Joly *et al.,* 1991; Joly & Oldstone, 1992). Therefore, assessment of a H-2K^d restricted anti-HSV response in S-130 infected cell lines may have been a useful measure of S-130 encoded H-2K^d function.

In vivo, H-2K^d antigen expression on the surfaces of primary sensory neurons did not result in accelerated clearance of infectious virus or viral DNA from ganglia of non-H-2K^d mice. As already discussed, the expression of uncomplexed, non-functional H-2K^d αCs or low cell-surface H-2K^d antigen expression might explain this result. Alternatively, it is possible that any effect of alloreactive CTLs was masked by the normally efficient CD8$^+$ T cell mediated clearance of HSV from ganglia (Simmons & Tscharke, 1992), which is presumed to be directed against viral antigens. Finally, killing of allogeneic cells by alloreactive CTLs requires not only recognition of cell-surface MHC-Ia antigens but also stabilization of cell to cell contact (see section 1.6.4). Several molecules are involved in this process, particularly ICAM-1. Absence of ICAM-1 can render an MHC-Ia expressing target cell less susceptible to recognition and lysis by CTLs (Gregory *et al.,* 1988; White *et al.,* 1994). Studies on neuroblastoma cell lines have shown a lack of constitutive ICAM-1 expression (Birdsall, 1991) which could not be upregulated with γ-IFN and it has been proposed that this may enable neurons to evade alloreactive CTL mediated lysis (White *et al.,* 1994). Further, lower expression of adhesion molecules such as ICAM-1 may allow Burkitt's lymphoma cells to escape EBV specific CTL surveillance (Gregory *et al.,* 1988). As a result of these studies, ICAM-1 expression in ganglia infected with S-130 was assessed.

ICAM-1 mRNA transcripts were shown by northern blot hybridization to be induced in ganglia productively infected with HSV. Cell-surface ICAM-1 was detected on ganglionic glial cells but not neurons 5 days after infection. Eight days after infection, ICAM-1 was detected on the surfaces of a substantial proportion of glial cells but only a small minority of ganglionic neurons. Failure to detect ICAM-1 on primary sensory neurons from HSV infected ganglia concurs with studies on neuroblastoma cells (Birdsall, 1991; White *et al.,* 1994) and further studies are required to determine whether failure to express ICAM-1 protects virally infected neurons against CTL attack.

In adoptive transfer experiments, expression of H-2K^d on the surfaces of infected cells had no measurable effect on virus clearance from the skin. Several factors must be taken into account when interpreting this result. Firstly, it was not formally proven that H-2^d restricted immune cells were generated, because there was no BALB/c recipient control. Secondly, there may have been rapid destruction of transferred cells by alloreactive CTLs, although this is unlikely because recipient mice were sublethally irradiated. Thirdly, data from humans suggest that virus clearance from the skin is dependent primarily on MHC class II rather than MHC class I restricted T-cells (Schmid & Rouse, 1992).

Others have shown that the level of HCMV promoter activity in HSV recombinant vectors decreases markedly in the first few days following infection and detection of long term expression has been problematic (Kaplitt *et al.,* 1991; Ho *et al.,* 1993; Smith *et al.,* 1994 b). Nevertheless, given the rapid turnover of MHC-Ia αC molecules (Neefjes *et al.,* 1992), the immunohistochemical detection of H-2K^d antigens in a small number of sensory neurons suggests that there may have been continued transcription from the H-2K^d expression cassette for at least 25 days after infection. In addition, the detection of H-2K^d antigen expression over this period suggests that there was prolonged expression of β_2m and TAPs. Indeed, Pereira *et al* (1994) showed that upregulated β_2m mRNA transcription is slow to decline after recovery from productive infection.

This thesis presents evidence that neurons *in vivo* are capable of expressing surface MHC-Ia antigens following infection with a recombinant HSV encoding a MHC-Ia αC (H-2K^d) expression cassette. Expression of β_2m, TAPs and ICAM-1 was shown to be upregulated in ganglia infected with HSV. An extension of these studies would be to determine whether CTLs have the ability to recognize and lyse HSV infected neurons expressing cell-surface MHC-Ia molecules *in vivo*. Given that CD8$^+$ T cells appear to play a non-cytolytic role in clearance of HSV from the PNS (Simmons & Tscharke, 1992), it is conceivable that neurons or glial cells may release factors that interfere with lytic CTL-neuronal interactions, preventing virally infected

neurons from being killed. If this were the case, culturing neurons *in vitro* may remove factors essential for this protective effect on neurons. Finally, it is clear that recombinant HSVs used *in vivo* are a powerful tool to study the molecular mechanisms of immune regulation in the PNS during viral infection.

APPENDIX 1-Abbreviations

+ve	positive
-ve	negative
% G+C	percentage guanosine and cytosine
αC	MHC-Ia heavy chain
αTIC	alpha transinducing complex
αTIF	alpha transinducing factor
β_2m	β_2-microglobulin
μCi	microcurie
μg	microgram
μl	microlitre
μm	micrometre
μM	micromolar
°C	degrees celsius
2ME	2-mercaptoethanol
ADCC	antibody dependent cellular cytotoxicity
APCs	antigen presenting cells
APES	aminopropyltriethoxysilane
ATP	adenosine-5'-triphosphate
bp	base pair
BgH	bovine growth hormone
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CAT	chloroamphenicol acetyl-transferase
cDNA	complementary DNA
cGMP	cyclic guanosine monophosphate
Ci	curie
cm	centimetre
CMC	carboxymethylcellulose
CMV	cytomegalovirus

CNS	central nervous system
cpm	counts per minute
CRE	cAMP response element
CSL	Commonwealth serum laboratories
CTL	cytotoxic T lymphocyte
CTP	cytidine-5'-triphosphate
CYT	cytoplasmic
DAB	3, 3'-diaminobenzidine
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'-deoxy-cytidine-5'-triphosphate
DDW	double distilled water
dGTP	2'-deoxy-guanosine-5'-triphosphate
DIG	digoxigenin
DIG-UTP	digoxigenin-11-rUTP
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease
dpm	decays per minute
DTT	dithiothreitol
dTTP	2'-deoxy-thymidine-5'-triphosphate
E	early
EBV	Epstein-Barr virus
EHV-1	equine herpesvirus type 1
ER	endoplasmic reticulum
Fab	fragment (ab) region of immunoglobulin
FCS	fetal calf serum
g	glycoprotein
x g	gravity
GAB	guanidine acetate buffer
GM	growth medium
GPI	glycosyl phospatidylinositol

GTP	guanosine-5'-triphosphate
HBS	HEPES buffered saline
H-2	mouse MHC
HCMV	human cytomegalovirus
HHV	human herpesvirus
HLA	human MHC
HPRT	human phosphoribosyl transferase
hr	hour
HSV	herpes simplex virus
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
i.v.	intravenous
ICAM-1	intercellular adhesion molecule-1
ICP	infected cell polypeptide
IE	immediate early
IFN	interferon
Ig	immunoglobulin
IHV-1	icatlurid herpesvirus 1
IL	interlukin
IR_L	inverted repeat flanking U_L
IR_S	inverted repeat flanking U_S
ISH	*in situ* hybridization
J	junction
kb	kilobase
kDa	kilodalton
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma herpesvirus
L-1	lumbar segment 1
L	late
Ld	leader
LAA	latency associated antigen

LAP1	latency asscoiated promoter element 1
LAP2	latency asscoiated promoter element 2
LAT	latency associated transcript
LCMV	lymphocytic choriomeningitis virus
LFA	lymphocyte function associated
LMP	low molecular weight polypeptide
M	molar
MCMV	murine CMV
mg	milligram
MHC	major histocompatibility complex
MHC-Ia	classical MHC class I molecules
MHC-Ib	non-classical MHC class I molecules
min	minute
ml	millilitre
MLC	mixed lymphocyte culture
mm	millimetre
mM	millimolar
MM	maintenance medium
moMLV	moloney leukemia virus
mRNA	messenger ribonucleic acid
ng	nanogram
NK	natural killer
nm	nanometre
NGS	normal goat serum
NRS	normal rabbit serum
NSS	normal swine serum
NSE	neuron specific enolase
OCT	optimum cutting temperature compound
Oct-1	octomer binding protein
ORF	open reading frame
ori	origin of replication

OVA	ovalbumin
p	plasmid
PAP	peroxidase anti-peroxidase
p.i.	post infection
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pfu	plaque forming unit
PLP	periodate-lysine paraformaldehyde
PNS	peripheral nervous system
pre-mRNA	precursor mRNA
prot A-SRBC	protein A coupled srbc
PRV	pseudorabies virus
PVP	polyvinyl pyrolidone
rATP	2'-ribose-adenosine-5'-triphosphate
rCTP	2'-ribose-cytidine-5'-triphosphate
RFC	rosette forming cell
rGTP	2'-ribose-guanosine-5'-triphosphate
RNA	ribonucleic acid
RNAse	ribonulease
rUTP	2'-ribose-uridine-5'-triphosphate
SDS	sodium doecyl sulphate
SEM	standard error of the mean
SRBC	sheep red blood cells
SRP	signal recognition pathway
SSC	standard saline citrate
SV	simian virus
T	thoracic
TAPs	transporters associated with antigen processing
TBS	tris buffered saline
TCA	trichloroacetic acid
TCR	T cell receptor

TK	thymidine kinase
TM	transmembrane
T_m	temperature at which 50% of nucleic acid hybrids dissociate
TR_L	terminal repeat flanking U_L
TR_S	terminal repeat flanking U_S
ts	temperature sensitive
U_L	long unique region
U_S	short unique region
UTP	uridine-5'-triphosphate
UV	ultraviolet
V	volt
VHS	virion host shut-off
Vmw	virion protein molecular weight
VP	virion polypeptide
VSV	vesicular stomatitis virus
VV	vaccinia virus
VZV	varicella-zoster virus
w/v	weight per volume
X-gal	5-bromo-chloro-3-idoyl-B-D-β-galactopyranoside

APPENDIX 2-Buffers and solutions

CSL water: Cell culture grade sterile deionized water (Commonwealth Serum Laboratories, Victoria, Australia)

Denaturation solution: 0.5M NaOH, 1.5M NaCl.

Denhardts solution (50x): 1mg/ml polyvinyl pyrrolidone (PVP), 1mg/ml bovine serum albumin (BSA), 1mg/ml ficoll 400.

DIG-11-UTP: 10mM digoxigenin-3-O-methylcarbonyl-E-amino-caproyl-[5-(3-aminoallyl)-uridine-5'-triphosphate].

Digoxigenin (DIG) buffer 1: 100mM Tris-HCl pH 7.5, 150mM NaCl.

DIG buffer 2: 1% (w/v) blocking reagent for nucleic acid hybridization (Boehringer Mannheim, Germany) dissolved in DIG buffer 1.

DIG buffer 3: 100mM Tris-HCl pH 9.5, 100mM NaCl, 50mM $MgCl_2$.

DNA tracking dye: 50% glycerol, 0.25% bromophenol blue.

Formal saline: 10% formalin in PBS.

Guanidine acetate buffer (GAB): 6M guanidine HCl, 200mM sodium acetate pH 5.5.

HDMEM: 20 mM HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid) buffered Dulbeco modified Eagle's medium (Gibco BRL), with 10mM $NaHCO_3$, 12 μg/ml penicillin, 16 μg/ml gentamicin, pH 7.2.

HDMEM-MM (maintenance medium): HDMEM + 1% FCS.

HDMEM-GM (growth medium): HDMEM + 10% FCS.

Hybridization buffer (5x) for *in situ* hybridization: 5xSSC, 500mM Tris-HCl pH 7.6, 50mM NaH_2PO_4, 50mM Na_2HPO_4, 0.1% ficoll, 0.1% PVP.

Hybridization solution for *in situ* hybridization: 50% deionized formamide (Davis *et al.,* 1986), 1xSSC, 1x hybridization buffer, 500 μg/ml sheared and denatured salmon sperm DNA, 0.5mg/ml tRNA, 20mM DTT, 1U/μl RNAsin (ribonuclease inhibitor, Promega, USA), DIG labelled riboprobe.

Hybridization solution for northern blot hybridization: 1x Denhardts solution, 5x SSC, 0.1% SDS, 50% deionized formamide, 100μg/ml sheared and denatured salmon sperm DNA, 10% dextran sulphate, $2.5x10^6$ cpm/ml denatured ^{32}P-labelled DNA probe.

Hybrdization solution for Southern blot hybridization: 3x Denhardts solution, 4x SSC, 0.5% SDS, 200μg/ml sheared and denatured salmon sperm DNA, 10mM Na_2HPO_4, 10mM NaH_2PO_4, 10% dextran sulphate, $2.5x10^6$ cpm/ml denatured ^{32}P-labelled DNA probe.

MOPS buffer (10x): 200mM MOPS, Free acid 3-[N-Morpholino] propane sulphonic acid (Sigma, USA), 50mM sodium acetate, 10mM EDTA, pH 7.0.

Neutralization solution: 3M NaCl, 0.5M Tris-HCl, pH 7.5.

Nitroblue tetrazolium chloride (NBT) solution: 75mg/ml NBT in 70% (v/v) dimethylformamide.

PBS (phosphate buffered saline): 140mM NaCl, 3mM KCl, 1mM KH_2PO_4, 8mM Na_2HPO_4 (Sambrook *et al.,* 1989).

PLP (periodate-lysine-paraformaldehyde): 10mM sodium periodate, 75mM lysine, 2% paraformaldehyde, 37mM phosphate buffer pH 7.4 (McLean & Nakane, 1974).

Prehybridization solution for northern blot hybridization: 5x Denhardts solution, 5xSSC, 0.1% SDS, 50% deionized formamide, 200μg/ml sheared and denatured salmon sperm DNA, 50mM Na_2HPO_4, 50mM NaH_2PO_4.

Prehybridization solution for Southern blot hybridization: 2x Denhardts solution, 6xSSC, 0.5% SDS, 200μg/ml sheared and denatured salmon sperm DNA, 10mM Na_2HPO_4, 10mM NaH_2PO_4.

Rapid haematoxylin: 110mM aluminium ammonium sulphate, 13mM haematoxylin, 1.5mM sodium iodate, 8mM citric acid, 450mM chloral hydrate.

RNA denaturation buffer: 500μl deionized formamide, 150μl 40% w/v formaldehyde solution, 100μl 10x MOPS buffer.

RNA tracking dye: 50% glycerol, 1X MOPS buffer, 0.25% bromophenol blue.

RPMI-MM (maintenance medium): 20mM HEPES buffered RPMI 1640 (Gibco BRL) with 10mM $NaHCO_3$, 12 μg/mL penicillin, 16 μg/ml gentamicin + 1% heat inactivated FCS.

RPMI-GM (growth medium): 20mM HEPES buffered RPMI 1640 (Gibco BRL) with 10mm $NaHCO_3$, 12 μg/ml penicillin, 16 μg/mL gentamicin + 10% heat inactivated FCS.

RPMI-GM-2ME: RPMI-GM + 10^{-5}M 2-mercaptoethanol.

SSC (20x): 3mM NaCl, 300mM trisodium citrate, pH 7.0.

TAE (10x): 400mM Tris, 200mM acetic acid, 10mM EDTA pH 8.0.

TBS: (Tris buffered saline) pH 7.4, 150mM NaCl, 50mM Tris.

TE: (10:1); 10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0, (20:50); 20mM Tris-HCl pH 8.0, 50mM EDTA pH 8.0.

Transcription Buffer (5x): 200mM Tris-HCl pH 7.5, 30mM $MgCl_2$, 10mM spermidine, 50mM NaCl.

Urea-SDS buffer: 7M urea, 350mM NaCl, 50mM Tris-HCl pH 7.5, 1mM EDTA pH 7.5, 0.2% SDS.

X-phosphate solution: 50 mg/ml 5-bromo-4-chloro-3-indolyl- phosphate 4-Toluidine salt (X-phosphate) in dimethylformamide.

BIBLIOGRAPHY

Abendroth, A. (1992). Construction and characterization of a recombinant HSV encoding a MHC class I gene. (Bachelor of Science, Honours dissertation), University of Adelaide, Australia.

Ace, C.I., McKee, T.A., Ryan, M., Cameron, J.M. & Preston, C.M. (1989). Construction and characterization of a herpes simplex virus type 1 mutant unable to transinduce immediate-early gene expression. *Journal of Virology* **63**, 2260-2269.

Adam, E., Kaufman, R.H., Mirkovic, R.R. & Melnick, J.L. (1979). Persistence of virus shedding in asymptomatic women after recovery from herpes genitalis. *Obstetrics and Gynecology* **54**, 171-174.

Ahluwalia, N., Bergeron, J.J.M., Wada, I., Degen, E. & Williams, D.B. (1992). The p88 molecular chaperone is identical to the endoplasmic reticulum membrane protein, calnexin. *Journal of Biological Chemistry* **267**, 10914-10918.

Aki, M., Shimbara, N., Takashina, M., Akiyama, K., Kagawa, S., Tamura, T., Tanashini, N., Yoshimura, T., Tanaka, K. & Ichihara. A. (1994). Interferon-γ induces different subunit organizations and functional diversity of proteasomes. *Journal of Biochemistry* **115**, 257-269.

Al-Saadi, S.A., Clements, G.B. & Subak-Sharpe, J.H. (1983). Viral genes modify herpes simplex latency both in mouse footpad and sensory ganglia. *Journal of General Virology* **64**, 1175-1179.

al-Kobaisi, M.F., Rixon, F.J., McDougall, I. & Preston, V.G. (1991). The herpes simplex virus UL33 gene product is required for the assembly of full capsids. *Virology* **180**, 380-388.

Allen, H., Fraser, J., Flyer, D., Calvin, S. & Flavell, R. (1986). β_2-microglobulin is not required for the cell surface expression of the murine class I histocompatibility antigen H-2D^b or of a truncated H-2D^b. *Proceedings of the National Academy of Sciences, USA* **83**, 7447-7451.

Alonso, S., Minty, A., Bourlet, Y. & Buckingham. M. (1986). Comparison of three actin-coding sequences in the mouse; evolutionary relationships between the actin genes of warm-blooded vertebrates. *Journal of Molecular Evolution* **23**, 11-22.

Ambroziak, J.A., Blackbourn, D.J., Herndier, B., Glogau, R.G., Gullett, J.H., McDonald, A.R., Lennette, E.T. & Levy, J.A. (1995). Herpesvirus-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. *Science* **268**, 582-583.

Andersen, J.K., Garber, D.A., Meaney, C.A. & Breakefield, X.O. (1992). Gene delivery transfer into mammalian CNS using herpes virus vectors: Long-term expression of bacterial *Lac Z* in neurons using the neuron-specific enolase promoter. *Human Gene Therapy* **3**, 487-499.

Anderson, K., Cresswell, P., Gammon, M., Hermes, J., Williamson, A., & Zweerink, H. (1991). Endogenously synthesized peptide with an endoplasmic reticulum signal sequence sensitizes antigen processing mutant cells to class-I restricted cell-mediated lysis. *Journal of Experimental Medicine* **174**, 489-492.

Andersson, M.L., Stam, N.J., Klein, G., Ploegh, H.L. & Masucci, M.G. (1991). Aberrant expression of HLA class-I antigens in Burkitt's lymphoma cells. *International Journal of Cancer* **47**, 544-550.

Andersson, M., Paabo, S., Nilsson, T. & Peterson, P.A. (1985). Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. *Cell* **43**, 215-222.

Andersson, M., McMichael, A. & Peterson, P.A. (1987). Reduced allorecognition of adenovirus-2 infected cells. *Journal of Immunology* **138**, 3960-3966.

Andrews, C.H. & Carmichael, E.A. (1930). A note on the presence of antibodies to herpesvirus in post-encephalitic and other human sera. *Lancet* **1**, 857-858

Androlewicz, M.J., Anderson, K.S., & Cresswell, P. (1993). Evidence that transporters associated with antigen processing translocate a major histocompatibility complex class I-binding peptide into the endoplasmic reticulum. *Proceedings of the National Academy of Sciences, USA* **90**, 9130-9134.

Androlewicz, M.J., Ortmann, B., Van-Endert, P.M., Spies, T. & Cresswell, P. (1994). Characteristics of peptide and major histocompatibility complex class I/beta 2-microglobulin binding to the transporters associated with antigen processing (TAP1 and TAP2). *Proceedings of the National Academy of Sciences, USA* **91**, 12716-12720.

Angerer, L.M., Stoler, M.H. & Angerer, R.C. (1987). *In Situ* Hybridization with RNA Probes: An Annotated Recipe. In: *In Situ Hybridization. Applications to Neurobiology*, pp. 42-70 Edited by Valentino, K.L., Eberwine, J.H. & Barchas, J.D. New York: Oxford University Press.

Arce-Gomez, B., Jones, E.A., Barnstable, C.J., Solomon, E. & Bodmer, W.F. (1978). The genetic control of HLA-A and B antigens in somatic cell hybrids; Requirement for $\beta_2$microglobulin. *Tissue Antigens* **11**, 96-104.

Arnold, D., Driscoll, J., Androlewicz, M., Hughes, E., Cresswell, P. & Spies, T. (1992). Proteasome subunits encoded in the MHC are not generally required for the processing of peptides bound by MHC class I molecules. *Nature* **360**, 171-173.

Attaya, M., Jameson, S., Martinez, C.K., Hermel, E., Aldrich, C., Forman, J., Lindahl, K.F., Bevan, M.J. & Monaco, J.J. (1992). Ham-2 corrects the class I antigen-processing defect in RMA-S cells. *Nature* **355**, 647-649.

Bacik, I., Cox, J.H., Anderson, R., Yewdell, J.W. & Bennik, J.R. (1994). TAP (transporter associated with antigen processing)- Independent presentation of endogenously synthesized peptides is enhanced by endoplasmic reticulum sequences located at the amino- but not carboxyl-terminus of the peptide. *Journal of Immunology* **152**, 381-387.

Bahram, S., Arnold, D., Bresnahan, M., Strominger, J.L. & Spies, T. (1991). Two putative subunits of a peptide pump encoded in the human major histocompatibility complex class II region. *Proceedings of the National Academy of Sciences, USA* **88**, 10094-10098.

Baringer, J.R. & Swoveland, P. (1973). Recovery of herpes simplex virus from human trigeminal ganglions. *The New England Journal of Medicine* **288**, 648-650.

Bartlett, P.F., Kerr, R.S.C.G. & Bailey, D.A. (1989). Expression of MHC antigens in the central nervous system. *Transplantation Proceedings* **21**, 3163-3165.

Barnes, P.D. & Grundy, J.E. (1992). Down-regulation of the class I HLA heterodimer and β_2-microglobulin on the surface of cells infected with cytomegalovirus. *Journal of General Virology* **73**, 2395-2403.

Barton, I.G., Kinghorn, G.R., Najem, S., Al-Omar, L.S. & Potter, C.W. (1982). Incidence of herpes simplex virus types 1 and 2 isolated from patients with herpes genitalis in Sheffield. *British Journal of Veneral Diseases* **58**, 44-47.

Bastian, F.O., Rabson, A.S., Yee, C.L. & Tralka, T.S. (1972). Herpesvirus hominis: isolation from human trigeminal ganglion. *Science* **178**, 306-307.

Bataille, D. & Epstein, A. (1994). Herpes simplex virus replicative concatamers contain L components in inverted orientation. *Virology* **203**, 384-388.

Batchelor, A.H. & O'Hare, P. (1990). Regulation and cell-type specific activity of a promoter located upstream of the latency-associated transcript of herpes simplex virus type 1. *Journal of Virology* **64**, 3269-3279.

Batchelor, A.H. & O'Hare, P. (1992). Localization of *cis*-acting sequence requirements in the promoter of the latency-associated transcript of herpes simplex virus type 1 required for cell-type-specific activity. *Journal of Virology* **66**, 3573-3582.

Batterson, W. & Roizman, B. (1983). Characterization of the herpes simplex virion-associated factor responsible for the induction of α genes. *Journal of Virology* **46**, 371-377.

Batterson, W., Furlong, D. & Roizman, B. (1983). Molecular genetics of herpes simplex virus. VII. Further characterization of a *ts* mutant defective in release of viral DNA and in other stages of viral reproductive cycle. *Journal of Virology* **45**, 397-407.

Battleman, D.S., Geller, A.I. & Chao, M.V. (1993). HSV-1 vector mediated gene transfer of the human nerve growth factor receptor p75 defines high-affinity NGF binding. *Journal of Neuroscience* **13**, 941-951.

Becker, Y., Asher, Y., Weinberg-Zahlering, E., Rabkin, S., Friedmann, A. & Kessler, E. (1978). Defective herpes simplex virus DNA: Circular and circular-linear molecules resembling rolling circles. *Journal of General Virology* **40**, 319-335.

Becker, Y., Dym, H. & Sarov, I. (1968). Herpes simplex virus DNA. *Virology* **36**, 184-192.

Beersma, M.F.C., Bijlmakers, M.J.E. & Ploegh, H.L. (1993). Human cytomegalovirus down-regulates HLA class I expression by reducing the stability of class I H chains. *Journal of Immunology* **151**, 4455-4464.

Beller, D.I. & Unanue, E.R. (1980). Ia antigens and antigen presenting function of thymic macrophages. *Journal of Immunology* **124**, 1433-1440.

Ben-Porat, T. & Kaplan, A.S. (1973). Replication-Biochemical Aspects. In *The Herpesviruses*. Edited by Kaplan, A.S. New York: Academic Press.

Ben-Porat, T. & Tokazewski, S. (1977). Replication of herpesvirus DNA. II. Sedimentation characteristics of newly synthesized DNA. *Virology* **79,** 292-301.

Berah, M., Hors, J. & Dausset, J. (1970). A study of HLA-A antigens in human organs. *Transplantation* **9**, 185-192.

Bergold, P.J., Casaccia-Bonnefil, P., Xiu-Liu, Z. & Federoff, H.J. (1993). Transsynaptic neuronal loss induced in hippocampal slice cultures by herpes simplex virus vector expressing the GluR6 subunit of the kainate receptor. *Proceedings of the National Academy of Sciences, USA* **90**, 6165-6169.

Berke, G. (1980). Interaction of cytolytic T lymphocytes and target cells. *Progress in Allergy* **27**, 69-133.

Bernstein, D.I. & Kappes J.C. (1988). Enhanced *in vitro* reactivation of latent herpes simplex virus from neural and peripheral tissues with hexamethylenebisacetamide. *Archives of Virology* **99,** 57-65.

Bierer, B.E., Sleckman, B.P., Ratnofsky, S.E. & Burakoff, S.J. (1989). The biological roles of CD2, CD4 and CD8 in T-cell activation. *Annual Reviews of Immunology* 7, 579-599.

Birdsall, H.H. (1991). Induction of ICAM-1 on human neural cells and mechanisms of neutrophil-mediated injury. *American Journal of Pathology* **139**, 1341-1350.

Biron, C.A., Byron, H.S. & Sullivan, J.L. (1989). Severe herpes virus infections in an adolescent without natural killer cells. *New England Journal of Medicine* **320**, 1731-1735.

Bix, M. & Raulet, D. (1992). Functionally conformed free class I heavy chains exist on the surface of β_2-microglobulin negative cells. *Journal of Experimental Medicine* **176**, 829-834.

Bjorkman, P.J., Saper, M., Samraoui, B., Bennett, W.S., Strominger, J.L. & Wiley, D.C. (1987 a). Structure of the human histocompatibility antigen, HLA-A2. *Nature* **329**, 506-512.

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L. & Wiley, D.C. (1987 b). The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* **329**, 512-518.

Block, T.M., Spivack, J.G., Steiner, I., Deshmane, S., McIntosh, M.T., Lirette, R.P. & Fraser, N.W. (1990). A herpes simplex virus type 1 latency-associated transcript mutant reactivates with normal kinetics from latent infection. *Journal of Virology* **64**, 3417-3426.

Block, T.M., Deshmane, S., Masonis, J., Maggioncalda, J., Valyi-Nagi, T. & Fraser, N.W. (1993). An HSV LAT null mutant reactivates slowly from latent infection and makes small plaques on CV-1 monolayers. *Virology* **192**, 618-630.

Bockenstedt, L.K., Goldsmith, M.A., Dustin, M., Olive, D., Springer, T.A. & Weiss, A. (1988). The CD2 ligand LFA-3 activates T cells but depends on the expression and function of the antigen receptor. *Journal of Immunology* **141**, 1904-1911.

Boenisch, T. (1980). Reference Guide Series 1. PAP/Immunoperoxidase. Santa Barbara: Dako Corporation.

Boes, B., Hengel, H., Ruppert, T., Multhaup, G., Koszinowski, U.H. & Kloetzel, P.-M. (1994). Interferon γ stimulation modulates the proteolytic activity and cleavage site preference of 20S mouse proteasomes. *Journal of Experimental Medicine* **179**, 901-909.

Bonneau, R.H. & Jennings, S.R. (1989). Modulation of acute and latent herpes simplex virus infection in C57BL/6 mice by adoptive transfer of immune lymphocytes with cytolytic activity. *Journal of Virology* **63**, 1480-1484.

Bonneau, R.H. & Jennings, S.R. (1990). Herpes simplex virus-specific cytolytic T lymphocytes restricted to a normally low responder H-2 allele are protective *in vivo*. *Virology* **174**, 599-604.

Booy, F.P., Newcomb, W.W., Trus, B.L., Brown, J.C., Baker, T.S. & Steven, A.C. (1991). Liquid-crystalline, phage-like packing of encapsidated DNA in herpes simplex virus. *Cell* **64**, 1007-1015.

Breakefield, X.O. & DeLuca, N.A. (1991). Herpes simplex virus for gene delivery to neurons. *The New Biologist* **3**, 203-218.

Brenner, M.B., Strominger, J.L. & Krangel, M.S. (1988). The $\gamma\delta$ T cell receptor. *Advances in Immunology* **43**, 133-192.

Brown, M.G., Driscoll, J. & Monaco, J.J. (1991). Structural and serological similarity of MHC-linked LMP and proteasome (multicatalytic proteinase) complexes. *Nature* **353**, 335-360.

Brown, M.G., Driscoll, J. & Monaco, J.J. (1993). MHC-linked low molecular mass polypeptide subunits define distinct subsets of proteasomes: implications for divergent function among distinct proteasome subsets. *Journal of Immunology* **151**, 1193-1204.

Browne, H., Smith, G., Beck, S. & Minson, T. (1990). A complex between the MHC class I homologue encoded by human cytomegalovirus and $\beta 2$ microglobulin. *Nature* **347**, 770-772.

Bruckner, R.C., Dutch, R.E., Zemelman, B.V., Mocarski, E.S. & Lehman, J.R. (1992). Recombination *in vitro* between herpes simplex virus type 1 -a-sequences. *Proceedings of the National Academy of Sciences, USA* **89**, 10950-10954.

Bublot, M., Lomonte, A.-S., LeQuarre, J.-C., Nicholas, J., Fleckenstein, B., Pastoret, P.-P. & Thiry, E. (1992). Genetic relatonships between bovine herpesvirus 4 and gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri. *Virology* **190**, 654-665.

Buchholz, D., Scott, P. & Shastri, N. (1995). Presentation without proteolytic cleavage of endogenous precursors in the MHC class I antigen processing pathway. *Journal of Biological Chemistry* **270**, 6515-6522.

Buchman, T.G., Roizman, B. & Nahmias, A.J. (1979). Demonstration of ex-ogenous genital reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA. *Journal of Infectious Diseases* **140**, 295-304.

Buckmaster, A.E., Scott, S.D., Sanderson, M.J., Boursnell, M.E.G., Ross, N.L.J. & Binns, M.M. (1988). Gene sequence and mapping data from Marek's disease virus and herpesvirus of turkeys: implications for herpesvirus classification. *Journal of General Virology* **69**, 2033-2042.

Buddingh, G.J., Schrum, D.I., Lanier, J.C. & Guidry, D.J. (1953). Studies on the natural history of herpes simplex infections. *Pediatrics* **11**, 595-610.

Bukowski, J.F. & Welsh, R.M. (1986). The role of natural killer cells and interferon in resistance to acute infection of mice with herpes simplex type 1. *Journal of Immunology* **136**, 3481-3485.

Burchett, S.K., Corey, L., Mohan, L., Westall, K.M., Ashley, R. & Wilson, C.B. (1992). Diminished interferon- γ and lymphocyte proliferation in neonatal and postpartum primary herpes simplex virus infection. *Journal of Infectious Diseases* **165**, 813-818.

Burgert, H-G. & Kvist, S. (1985). An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell* **41**, 987-997.

Burgert, H-G., Maryanski, J.L. & Kvist, S. (1987). E3/19K protein of adenovirus type 2 inhibits lysis of cytolytic T lymphocytes by blocking cell-surface expression of histocompatibility class I antigens. *Proceedings of the National Academy of Sciences, USA* **84**, 1356-1360.

Burnet, F.M. & Williams, S.W. (1939). Herpes simplex: a new point of view. *The Medical Journal of Australia* **1**, 637-642.

Cabrera, C.V., Wohlenberg, C., Openshaw, H., Rey-Mendez, M., Puga, A. & Notkins, A.L. (1980). Herpes simplex virus DNA sequences in the CNS of latently infected mice. *Nature (London)* **288**, 288-290.

Cai, W., Gu, B. & Person, S. (1988). Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. *Journal of Virology* **62**, 2596-2604.

Cai, W. & Schaffer, P.A. (1989). Herpes simplex virus type 1 ICP0 plays a critical role in the *de novo* synthesis of infectious virus following transfection of viral DNA. *Journal of Virology* **63**, 4579-4589.

Campadelli-Fiume, G., Arsenakis, M., Farabegoli, F. & Roizman, B. (1988). Entry of herpes simplex virus 1 in BJ cells that constitutively express viral glycoprotein D is by endocytosis and results in the degradation of the virus. *Journal of Virology* **62**, 159-167.

Campadelli-Fiume, G., Stripe, D., Boscaro, A., Avitabile, E., Foa-Tomasi, P., Barker, D. & Roizman, B. (1990). Glycoprotein C-dependent attachment to herpes simplex virus to susceptible cells leading to productive infection. *Virology* **178**, 213-222.

Campbell, M.E.M., Palfreyman, J.W. & Preston, C.M. (1984). Identification of herpes simplex virus DNA sequences which encode a *trans*-acting polypeptide responsible for stimulation of immediate early transcription. *Journal of Molecular Biology* **180**, 1-19.

Campbell, A.E., Slater, J.S., Cavanaugh, V.J. & Sternberg, R.M. (1992). An early event in murine cytomegalovirus replication inhibits presentation of cellular antigens to cytotoxic T lymphocytes. *Journal of Virology* **66**, 3011-3017.

Campbell, A.E. & Slater, J.S. (1994). Down-regulation of major histocompatibility complex class I synthesis by murine cytomegalovirus early gene expression. *Journal of Virology* **68**, 1805-1811.

Cantin, E.M., Hinto, D.R., Chen, J. & Openshaw, H. (1995). Gamma interferon expression during acute and latent nervous system infection by herpes simplex virus type 1. *Journal of Virology* **69**, 4898-4905.

Cerundolo, V., Alexander, J., Anderson, K., Lamb, C., Cresswell, P., McMichael, A., Gotch, F. & Townsend, A.C. (1990). Presentaion of viral antigens controlled by a gene in the MHC. *Nature* **345**, 449-456.

Cerundolo, V., Elliott, T., Elvin, J., Bastin, J., Rammensee, H.G. & Townsend, A.R.M. (1991). The binding affinity and dissociation rates of peptides for class I major histocompatibility complex molecules. *European Journal of Immunology* **21**, 2069-2075.

Cesarman, E., Chang, Y., Moore, P.S., Said, J.W. & Knowles, D.M. (1995). Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *New England Journal of Medicine* **332**, 1186-1191.

Challberg, M. (1986). A method for identifying the viral genes required for herpesvirus DNA replication. *Proceedings of the National Academy of Sciences, USA* **83**, 9094-9098.

Chan, W.L., Ziltener, H.J. & Liew, F.Y. (1990). Interleukin-3 protects mice from acute herpes simplex virus infection. *Immunology* **71**, 358-363.

Chang, J.Y., Johnson, E.M., & Olivo, P.D. (1991). A gene delivery/recall system for neurons which utilizes ribonucleotide reductase-negative herpes simplex viruses. *Virology* **185**, 437-440.

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M. & Moore, P.S. (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **266**, 1865-1869.

Chen, C. & Okayama, H. (1987). High-efficiency transformation of mammalian cells by plasmid DNA. *Molecular & Cellular Biology* **7**, 2742-2752.

Chen, E., Karr, R.W., Frost, I.P., Gonwas, T.A. & Ginder, G.D. (1986). Gamma interferon and 5-azactidine cause transcriptional elevation of class I major histocompatibility complex gene expression in K562 leukemia cells in the absence of differentiation. *Molecular & Cellular Biology* **6**, 1698-1705.

Cheong, W.K., Thirumoorthy, T., Dorai Singham, S. & Ling, A.E. (1990). Clinical and laboratory study of the first episode genital herpes in Singapore. *International Journal of STD and AIDS* **1**, 195-198.

Cheung, A.K. (1989). Detection of pseudorabies virus transcripts in trigeminal ganglia of latently infected swine. *Journal of Virology* **63**, 2903-2913.

Cheung, A.K. (1990). The BamHI J fragment (0.706 to 0.737 map units) of pseudorabies virus is transcriptionally active during viral replication. *Journal of Virology* **64**, 977-983.

Christinck, R.E., Luscher, M.A., Barber, B.H. & Williams, D.B. (1991). Peptide binding to class I MHC on living cells and quantitation of complexes required for CTL lysis. *Nature* **352**, 67-70.

Ciechanover, A. (1994). The ubiquitin-proteasome proteolytic pathway. *Cell* **79**, 13-21.

Claoue, C.M.P., Hodges, T.J., Darville, J.M., Hill, T.J., Blyth, W.A. & Easty, D.L. (1990). Possible latent infection with herpes simplex virus in the mouse eye. *Journal of General Virology* **71**, 2385-2390.

Clements, G.B. & Subak-Sharpe, J.H. (1988). Herpes simplex virus type 2 establishes latency in the mouse footpad. *Journal of General Virology* **69**, 375-383.

Clements, G.B. & Stow, N.D. (1989). A herpes simplex virus type 1 mutant containing a deletion within immediate early gene 1 is latency-competent in mice. *Journal of General Virology* **70**, 2501-2506.

Clements, G.B. & Jamieson, G.B. (1989). Reactivation of latent herpes simplex virus-1 (HSV) from mouse footpad cells demonstrated by *in situ* hybridization. *Archives of Virology* **104**, 95-106.

Clements, J.B. (1993). Herpesviridae: properties of viruses. In: *Principles of Bacteriology, Virology and Immunity,* pp. 416-429, 8th Ed., vol 4. Edited by Collier, L.H. & Timbury, M.C. London: Topley, Wilsons.

Clevers, H., Alarcon, B., Wileman, T. & Terhorst, C. (1988). The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annual Reviews of Immunology* **6**, 629-662.

Coen, D.M., Kosz-Vnenchak, M., Jacobson, J.G., Leib, D.A., Bogard, C.L., Schaffer, P.A., Tyler, K.L. & Knipe, D.M. (1989). Thymidine kinase-negative herpes simplex virus mutants establish latency in mouse trigeminal ganglia but do not reactivate. *Proceedings of the National Academy of Sciences, USA* **86**, 4736-4740.

Cohen, J. (1994). Controversy: Is KS really caused by a new herpesvirus. *Science* **268**, 1847-1848.

Coligan, J.E., Kindt, T.T., Uehara, H., Martinko, J. & Nathenson, S.G. (1981). Primary structure of a murine transplantation antigen. *Nature* **291**, 35-39.

Cone, R.D. & Mulligan, R.C. (1984). High efficiency gene transfer into mammalian cells: Generation of helper-free recombination retrovirus with broad mammalian host range. *Proceedings of the National Academy of Sciences, USA* **81**, 6349-6353.

Conley, A.J., Knipe, D.M., Jones, P.C. & Roizman, B. (1981). Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by *in vitro* mutagenesis and defective in DNA synthesis and accumulation of γ polypeptides. *Journal of Virology* **37**, 191-206.

Cook, M.L. & Stevens, J.G. (1973). Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. *Infection and Immunity* **7**, 272-288.

Cook, M.L., Bastone, V.B. & Stevens, J.G. (1974). Evidence that neurons harbor latent herpes simplex virus. *Infection and Immunity* **9**, 946-951.

Cook, M.L. & Stevens, J.G. (1976). Latent herpetic infections following experimental viraemia. *Journal of General Virology* **31**, 75-80.

Cook, S.D., Batra, S.K. & Brown, S.M. (1987). Recovery of herpes simplex virus from the corneas of experimentally infected rabbits. *Journal of General Virology* **68**, 2013-2017.

Cook, S.D., Hill, J.M., Lynas, C. & Maitland, N.J. (1991). Latency associated transcripts in corneas and ganglia of HSV-1 infected rabbits. *British Journal of Opthalmology* **75**, 644-648.

Corey, L., Adams, H.G., Brown, Z.A. & Holmes, K.K. (1983). Genital herpes simplex virus infections: clinical manifestations, course and complications. *Annals of Internal Medicine* **98**, 958-972.

Corey, L. & Spear, P.D. (1986). Infections with herpes simplex viruses. *The New England Journal of Medicine* **314**, 686-691, 749-757.

Corey, L. (1988). First-episode, recurrent, and asymptomatic herpes simplex infections. *Journal of the American Academy of Dermatology* **18**, 169-172.

Cosman, D., Kress, M., Khoury, G. & Jay, G. (1982). Tissue specific expression of an unusual H-2 (class I)-related gene. *Proceedings of the National Academy of Sciences, USA* **79**, 4947-4951.

Costa, R.H., Draper, K.G., Kelly, T.J. & Wagner, E.K. (1985). An unusual spliced herpes simplex virus type 1 transcript with sequence homology to Epstein-Barr virus DNA. *Journal of Virology* **54**, 317-328.

Costanzo, F., Campadelli-Fiume, G., Foa-Tomasi, L. & Cassai, E. (1977). Evidence that herpes simplex virus DNA is transcribed by cellular RNA polymerase B. *Journal of Virology* **21**, 996-1001.

Coupar, B.E.H., Andrew, M.E., Boyle, D.B. & Blanden, R.V. (1986). Immune response to H-2K^d antigen expressed by recombinant vaccinia virus. *Proceedings of the National Academy of Sciences, USA* **83**, 7879-7882.

Cowing, C., Schwartz, B.C. & Dickler, H.B. (1979). Macrophage Ia antigens. I. Macrophage populations differ in their expression of Ia antigens. *Journal of Immunology* **120**. 378-384.

Cox, K.H., DeLeon, D.V., Angerer, L.M. & Angerer, R.C. (1984). Detection of mRNAs in sea urchin embryos by *in situ* hybridization using asymmetric RNA probes. *Developmental Biology* **101**, 485-502.

Craighead, J.E., Kanish, R.E. & Almeida, J.D. (1972). Non-viral microbodies with viral antigenicity produced in cytomegalovirus-infected cells. *Journal of Virology* **10**, 766-775.

Cresswell, P., Turner, M.J. & Strominger, J.L. (1973). Papain-solubilized HLA-A antigens from cultured human lymphocytes contain two peptide fragments. *Proceedings of the National Academy of Sciences, USA* **70**, 1603-1607.

Croen, K.D., Ostrove, J.M., Dragovic, L.J., Smialek, J.E. & Straus, S.E. (1987). Latent herpes simplex virus in human trigeminal ganglia. Detection of an immediate early gene "anti-sense" transcript by *in situ* hybridization. *The New England Journal of Medicine* **317**, 1427-1432.

Cunningham, A.L. & Merigan, T.C. (1983). γ-Interferon production appears to predict time of recurrence of herpes labialis. *Journal of Immunology* **130**, 2397-2400.

Daar, A., Fuggle, S., Fabre, J., Ting, A. & Morris, P. (1984). The detailed distribution of HLA-A, B, C antigens in normal human organs. *Transplantation* **38**, 287-292.

Dal Porto, J., Johansen, R.E., Catipovic, B., Parfiit, D.J., Tureson, D., Gether, U., Kozlowski, S., Fearon, D.T. & Schneck, J.P. (1993). A soluble divalent class I major histocompatibility complex molecule inhibits alloreactive T cells at nanomolar concentrations. *Proccedings of the National Academy of Sciences, USA* **90**, 6671-6675.

Darlington, R.W. & Moss, L.H.I.I. (1969). The envelope of herpesvirus. *Progress in Medical Virology* **11**, 16-45.

Darlington, R.W. & Granoff, A. (1973). Replication-Biological Aspects. In *The Herpesviruses*, pp. 93-132. Edited by Kaplan, A.S. New York: Academic Press.

Davar, G., Kramer, M.F., Garber, D., Roca, A.L., Andersen, J.K., Berrin, W., Coen, D.M., Kosz-Vnenchak, M., Knipe, D.M., Breakefield, X.O. & Isacson, O. (1994). Comparative efficacy of expression of genes delivered to mouse sensory neurons with herpes virus vectors. *Journal of Comparative Neurology* **339**, 3-11.

David, V., Hochstenbach, F., Rajagopalan, S. & Brenner, M.B. (1992). Interaction with newly synthesized and retained proteins in the endoplasmic reticulum suggests a chaperone function for human integral membrane protein IP90 (calnexin). *Journal of Biological Chemistry* **268**, 9585-9592.

Davis, L.G., Dibner, M.D. & Battey, J.F. (1986). *Basic Methods in Molecular Biology*. 1st edition. New York: Elsevier.

Davis, M.M. & Chien, Y-H. (1993). Topology and affinity of T-cell receptor mediated recognition of peptide-MHC complexes. *Current Opinion in Immunology* **5**, 45-49.

Davison, A.J. & Wilkie, N.M. (1981). Nucleotide sequence of the joint between L and S sequences of herpes simplex virus types 1 and 2. *Journal of General Virology* **55**, 315-331.

Davison, A.J. & Wilkie, N.M. (1983 a). Inversion of the two segments of the herpes simplex virus genome in intertypic recombinants. *Journal of General Virology* **64**, 1-18.

Davison, A.J. & Wilkie, N.M. (1983 b). Either orientation of the L segment of the herpes simplex virus genome may participate in the production of viable intertypic recombinants. *Journal of General Virology* **64**, 247-250.

Dawson, C.R. & Togni, B. (1976). Herpes virus eye infections: Clinical manifestations, pathogenesis and management. *Surveys in Opthalmology* **21**, 121-125.

Deatly, A.M., Spivack, J.G., Lavi, E. & Fraser, N.W. (1987). RNA from an immediate early region of the type 1 herpes simplex virus genome is present in the trigeminal ganglia of latently infected mice. *Proceedings of the National Academy of Sciences, USA* **84**, 3204-3208.

Deatly, A.M., Spivack, J.G., Lavi, E., O'Boyle, D.R. & Fraser, N.W. (1988). Latent herpes simplex virus type 1 transcripts in peripheral and central nervous system tissues of mice map to similar regions of the viral genome. *Journal of Virology* **62**, 749-756.

Deb, S. & Doelberg, M. (1988). A 67-base-pair segment from the ori-S region of herpes simplex virus type 1 encodes origin function. *Journal of Virology* **62**, 2516-2519.

Debroy, C., Pederson, N. & Person, S. (1985). Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. *Virology* **145**, 36-48.

Deckhut, A.M., Lippolis, J.D. & Tevethia, S.S. (1992). Comparative analysis of core amino acid residues of H-2D (b)-restricted cytotoxic T-lymphocyte recognition epitopes in simian virus 40 T antigen. *Journal of Virology* **66**, 440-447.

Degen, E. & Williams, D.B. (1991). Participation of a novel 88 kD protein in the biogenesis of murine class I histocompatibility molecules. *Journal of Cell Biology* **112**, 1099-1115.

Degen, E., Cohen-Doyle, M.F. & Williams, D.B. (1992). Efficient dissociation of the p88 chaperone from MHC class I molecules requires both β_2-microglobulin and peptide. *Journal of Experimental Medicine* **175**, 1653-1661.

Deinhardt, F., Falk, L.A. & Wolfe, L.G. (1974). Simian herpesviruses and neoplasia. *Advances in Cancer Research* **19**, 167-205.

Deiss, L.P. & Frenkel, N. (1986). Herpes simplex virus amplicon: cleavage of concatameric DNA is linked to packaging and involves amplification of the terminally reiterated *a* sequence. *Journal of Virology* **57**, 933-941.

Deiss, L.P., Chou, J. & Frenkel, N. (1986). Functional domains within the *a* sequence involved in the cleavage-packaging of herpes simplex virus DNA. *Journal of Virology* **59**, 605-618.

Del Val, M., Munch, K., Reddehase, M.J. & Koszinowski, U.H. (1989). Presentation of cytomegalovirus immediate-early antigens to cytotoxic T lymphocytes is selectively blocked by viral genes expressed in the early phase. *Cell* **58**, 305-315.

Del Val, M., Hengel, H., Hacker, H., Hartlaub, U., Ruppert, T., Kucin, P. & Koszinowski, U.H. (1992). Cytomegalovirus prevents antigen presentation by blocking the transport of peptide-loaded major histocompatibility complex class I molecules into the medial-Golgi compartment. *Journal of Experimental Medicine* **176**, 729-739.

Delius, H. & Clements, J.B. (1976). A partial denaturation map of herpes simplex virus type 1 DNA: evidence for inversions in the unique DNA regions. *Journal of General Virology* **33**, 125-133.

DeLuca, N., Bzik, D.J., Bond, V.C., Person, S. & Snipes, W. (1982). Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion and production of glycoprotein gB (VP7). *Virology* **122**, 411-423.

DeLuca, N.A., & Schaffer, P.A. (1985). Activation of immediate-early, early and late promoters by temperature-sensitive and wild-type forms of herpes simplex virus type 1 protein ICP4. *Molecular & Cellular Biology* **5**, 1997-2008.

DeMars, R., Rudersdorf, R., Chang, C., Peterson, J., Strandtmann, J., Korn, N., Sidwell, B. & Orr, H. (1985). Mutations that impair a post transcriptional step in the expression of HLA-A and -B antigens. *Proceedings of the National Academy of Sciences, USA* **82**, 8183-8187.

Desai, P.J., Schaffer, P.A. & Minson, A.C. (1988). Excretion of noninfectious virus particles lacking glycoprotein H by a temperature-sensitive mutant of herpes simplex virus type 1: evidence that gH is essential for virion infectivity. *Journal of General Virology* **69**, 1147-1156.

Deshmane, S.L. & Fraser, N.W. (1989). During latency, herpes simplex virus type 1 DNA is associated with nucleosomes in a chromosome structure. *Journal of Virology* **63**, 943-947.

Desrosiers, R.C., Kamine, J., Bakker, A., Silva, D., Woyckik, R.P., Sakai, D.D. & Rotmann, F.M. (1985). Synthesis of bovine growth hormone in primates by using herpesvirus vector. *Molecular and Cellular Biology* **5**, 2796-2803.

Deverson, E.R., Gow, I.R., Coadwell, W.J., Monaco, J.J., Butcher, G.W. & Howard, J.C. (1990). MHC class II region encoding proteins related to the multidrug resistance family of membrane transporters. *Nature* **348**, 738-741.

Devi-Rao, G., Goodart, S.A., Hecht, L.M., Rochford, R., Rice, M.K. & Wagner, E.K. (1991). Relationship between polyadenylated and nonpolyadenylated herpes simplex virus type 1 latency-associated transcripts. *Journal of Virology* **65**, 2179-2190.

Dick, L.R., Moomaw, C.R., Dematino, G.N. & Slaughter, C.A. (1991). Degradation of oxidized insulin B chain by the multiproteinase complex macroplain (Proteasome). *Biochemistry* **30**, 2725-2734.

Dick, L.R., Aldrich, C., Jameson, S.C., Mosman, C.R., Pramanik, B.C., Kuyler Doyle, C., DeMartino, G.N., Bevan, M.J., Forman, J.M. & Slaughter, C.A. (1994). Proteolyitc processing of ovalbumin and β-galactosidase by the proteasome to yield antigenic peptides. *Journal of Immunology* **152**, 3884-3894.

Dillard, S.H., Cheatham, W.J. & Moses, H.L. (1972). Electron microscopy of zosteriform herpes simplex infection in the mouse. *Laboratory Investigations* **26**, 391-402.

Dobberstein, B., Garoff, H., Warren, G. & Robinson, P.J. (1979). Cell free synthesis and membrane insertion of mouse H-2D^{d} histocompatibility antigens and β2-microglobulin. *Cell* **17**, 759-769.

Dobson, A.T., Sederati, F., Devi-Rao, G., Flanagan, W.M., Farrell, M.J., Stevens, J.G., Wagner, E.K. & Feldman, L.T. (1989). Identification of the latency-associated transcript promoter by expression of rabbit beta-globin mRNA in mouse sensory nerve ganglia latently infected with a recombinant herpes simplex virus. *Journal of Virology* **63**, 3844-3851.

Dobson, A.T., Margolis, T.P., Sedarati, F., Stevens, J.G. & Feldman, L.T. (1990). A latent, non-pathogenic HSV-1 derived vector stably expresses beta-galactosidase in mouse neurons. *Neuron* **5**, 353-360.

Doerig, C., Pizer, L.I. & Wilcox, C.L. (1991). An antigen encoded by the latency associated transcript in neuronal cell cultures latently infected with herpes simplex virus type 1. *Journal of Virology* **65**, 2724-2727.

Dowdle, W.R., Nahmias, A.J., Harwell, R.W. & Pauls, F.P. (1967). Association of antigenic type of herpesvirus hominis with site of viral recovery. *The Journal of Immunology* **99**, 974-980.

Drew, P.D., Lonergan, M., Goldstein, M.E., Lampson, L.A., Ozato, K. & McFarlin, D.E. (1993). Regulation of MHC class I and β_2-microglobulin gene expression in human neuronal cells. *Journal of Immunology* **150**, 3300-3310.

Driscoll, J., Brown, M., Finley, D. & Monaco, J.J. (1993). MHC-linked gene products specifically alter peptidase activities of the proteasome. *Nature* **365**, 262-264.

Dubbs, D.R. & Kit, S. (1964). Mutant strains of herpes simplex deficient in thymidine kinase-inducing activity. *Virology* **22**, 493-502.

During, M.J., Naegele, J., O'Malley, K. & Geller, A.I. (1994). Long term behavioural recovery in a model of Parkinson's disease by intrastriatal expression of tyrosine hydroxylase. *Science* **266**, 1399-1403.

Dustin, M.L. & Springer, T.A. (1989). T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* **341**, 619-624.

Dustin, M.L., Olive, D. & Springer, T.A. (1989). Correlation of CD2 binding and functional properties of multimeric and monomeric lymphocyte function associated antigen-3. *Journal of Experimental Medicine* **169**, 503-517.

Dustin, M.L. & Springer, T.A. (1991). Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. *Annual Reviews in Immunology* **9**, 27-66.

Dutch, R.E., Bruckner, R.C., Mocarski, E.S. & Lehman, I.R. (1992). Herpes simplex virus type 1 recombination: Role of DNA replication and viral -a- sequences. *Journal of Virology* **66**, 277-285.

Edidin, M. (1972). The tissue distribution and cellular location of transplantation antigens. In: *Transplantation Antigens*, pp 125-140. Edited by Kahan, B. & Reisfeld, B. New York: Academic Press.

Edwards, R.H., Selby, M.J. & Rutter, W.J. (1986). Differential RNA splicing predicts two distinct nerve growth factor precursors. *Nature* **319**, 784-787.

Efstathiou, S., Minson, A.C., Field, H.J., Anderson, J.R. & Wildy, P. (1986). Detection of herpes simplex virus-specific DNA sequences in latently infected mice and in humans. *Journal of Virology* **57**, 446-455.

Efstathiou, S., Kemp, S., Darby, G. & Minson, A.C. (1989). The role of herpes simplex virus type 1 thymidine kinase in pathogenesis. *Journal of General Virology* **70**, 869-879.

Eisenlohr, L.C., Bacik, I., Bennik, J.R., Bernstein, K. & Yewdell, J.W. (1992). Expression of membrane proteases enhances presentation of endogenous antigens to MHC class I-restricted T lymphocytes. *Cell* **71**, 963-972.

Ekwo, E., Wong, Y.W. & Myers, M. (1979). Asymptomatic cervicovaginal shedding of herpes simplex virus. *American Journal of Obstetrics and Gynaecology* **134**, 102-103.

Elliott, T., Cerundolo, V., Elvin, J. & Townsend, A. (1991). A peptide induced conformational change of the class I heavy chain. *Nature* **351**, 402-406.

Epstein, M.A. (1962). Observations on the mode of release of herpes virus from infected HeLa cells. *Journal of Cell Biology* **12**, 589-597.

Erb, P. & Feldmann, M. (1975). The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T-cell interaction for helper cell induction with soluble antigens. *Journal of Experimental Medicine* **142**, 460-472.

Everett, R.D. (1986). The products of herpes simplex virus type 1 (HSV-1) immediate early genes 1, 2 and 3 can activate HSV-1 gene expression in *trans*. *Journal of General Virology* **67**, 2507-2513.

Everett, R.D. (1989). Construction and characterization of herpes simplex virus mutants with defined lesions in immediate early gene 1. *Journal of General Virology* **70**, 1185-1202.

Falk, K., Rotzschke, O., Stevanovic, S., Jung, G. & Rammensee, H.G. (1991). Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* **351**, 290-296.

Falk, K. & Rotzschke, O. (1993). Consensus motifs and peptide ligands of MHC class I molecules. *Seminars in Immunology* **5**, 81-94.

Fareed, M.U. & Spivack, J.G. (1994). Two open reading frames (ORF1 and ORF2) within the 2.0 kilobase latency-associated transcript of herpes simplex virus type 1 are not essential for reactivation from latency. *Journal of Virology* **68**, 8071-8081.

Farrell, M.J., Dobson, A.T. & Feldman, L.T. (1991). Herpes simplex virus latency-associated transcript is a stable intron. *Proceedings of the National Academy of Sciences, USA* **88**, 790-794.

Farrell, M.J., Margolis, T.P., Gomes, W.A. & Feldman, L.T. (1994). Effect of the transcription start region of the herpes simplex virus type 1 latency-associated transcript promoter on expression of productively infected neurons *in vivo*. *Journal of Virology* **68**, 5337-5343.

Federoff, H.J., Geschwind, M.A., Geller, A.I. & Kessler, J.A. (1992). Expression of nerve growth factor *in vivo* from a defective herpes simplex virus 1 vector prevents effects of axotomy on sympathetic ganglia. *Proceedings of the National Academy of Sciences, USA* **89**, 1636-1640.

Fehling, H.J., Swat, W., Laplace, C., Kuhn, R., Rajewsky, K., Muller, U. & vonBoehmer, H. (1994). MHC class I expression in mice lacking the proteasome subunit LMP-7. *Science* **265**, 1234-1237.

Feinberg, A.P. & Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6-13.

Field, H.J. & Wildy, P. (1978). The pathogenicity of thymidine kinase-deficient mutants of herpes simplex virus in mice. *Journal of Hygiene* **81**, 267-277.

Fitzgerald-Bocarsly, P., Howell, D.M., Pettera, L., Tehrani, S. & Lopez, C. (1991). Immediate early gene expression is sufficient for induction of natural killer cell indicated lysis of herpes simplex virus type 1 infection fibroblasts. *Journal of Virology* **65**, 3151-3160.

Flaherty, L. (1981). The role of the MHC in immunology, pp 33-57. Edited by M.Dorf. New York: Garland STPM.

Flavell, R.A., Allen, H., Huber, B., Wake, C. & Widera, G. (1985). Organization and expression of the MHC of the C57Black/10 mouse. *Immunological Reviews* **84**, 29-50.

Flavell, R.A., Allen, H., Burkly, L.C., Sherman, D.H., Waneck, G.L. & Widera, G. (1986). Molecular biology of the H-2 histocompatibility complex. *Science* **233**, 437-443.

Foerster, O. (1933). The dermatomes in man. *Brain* 56, 1-39.

Forrester, A., Farrell, H., Wilkinson, G., Kaye, J., Davis-Poynter, N. & Minson, T. (1992). Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. *Journal of Virology* **66**, 341-348.

Forss-Petter, S., Danielson, P.E., Catsicas, S., Battenberg, E., Price, J., Neerenberg, M. & Sutcliffe, J.G. (1990). Transgenic mice expressing β-galactosidase in mature neurons under neuron-specific enolase promoter control. *Neuron* **5**, 187-197.

Foster, C.S., Opremcak, E.M. & Tolchin, N. (1989). Evidence for the potential influence of cyclic nucleotides on maintenance of or reactivation from latency of herpes simplex virus in trigeminal ganglionic neurons. *Acta Opthalmology (supplement)* **192**, 142-144.

Frank, A., Andiman, W.A. & Miller, G.A. (1976). Epstein-Barr virus and non-human primates: natural and experimental infections. *Advances in Cancer Research* **23**, 171-201.

Frehlinger, J.A., Niederhuber, J.E., David, C.S. & Shreffler, D.C. (1974). Evidence for the expression of Ia (H-2 associated) antigens on thymus-derived lymphocytes. *Journal of Experimental Medicine* **140**, 1273-1284.

Fremont, D.H., Matsumura, M., Stura, E.A., Peterson, P.A. & Wilson, I.A. (1992). Crystal structure of two viral peptides in complex with murine MHC class I H-2K^b. *Science* **257**, 919-927.

Frenkel, N., Spaete, R.R., Vlazny, D.A., Deiss, L.P. & Locker, H. (1982). The herpes simplex virus amplicon. A novel animal-virus cloning vector. In *Eukaryotic Viral Vectors*. Edited by Gluzman, Y. New York: Cold Spring Harbor Laboratory Press.

Frenkel, N., Schirmer, E.C., Wyatt, L.S., Katsafanas, G., Roffman, E., Danovich, R.M. & June, C.H. (1990). Isolation of a new herpesvirus from human CD4$^+$ T cells. *Proceedings of the National Academy of Sciences, USA* **87**, 748-752.

Fruh, K., Ahn, K., Djaballah, H., Sempe, P., van Endert, P.M., Tampe, R., Peterson, P.A. & Yang, Y. (1995). A viral inhibitor of peptide transporters for antigen presentation. *Nature* **375**, 415-418.

Fueta, Y., Kita, T. & Mita, T. (1989). Immunohistological detection of H-2 antigens *in vivo* induced by IFN-β in mouse brain tissues. *Basic Applied Histochemistry* **33**, 131-137.

Fuller, A.O. & Spear, P.G. (1987). Anti-glycoprotein D antibodies that permit adsorption but block infection by herpes simplex virus 1 prevent virion-cell fusion at the cell surface. *Proceedings of the National Academy of Sciences, USA* **84**, 5454-5458.

Fuller, A.O., Santos, R.E. & Spear, P.G. (1989). Neutralizing antibodies specific for glycoprotein H of herpes simplex virus permit viral attachment to cells but prevent penetration. *Journal of Virology* **63**, 3435-3443.

Fuller, A.O. & Lee, W.C. (1992). Herpes simplex virus type 1 entry through virus-cell interactions requires different roles of gD and gH in penetration. *Journal of Virology* **66**, 5002-5012.

Furlong, D., Swift, H. & Roizman, B. (1972). Arrangement of herpesvirus deoxyribonucleic acid in the core. *Journal of Virology* **10**, 1071-1074.

Futcher, A.B. (1986). Copy number amplification of the 2μm circle plasmid of Saccharomyces cerevisiae. *Journal of Theoretical Biology* **119**, 197-204.

Gaczynska, M., Rock, K.L. & Goldberg, A.L. (1993). Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature* **365**, 264-267.

Gaczynska, M., Rock, K.L., Spies, T. & Goldberg, A.L. (1994). Peptidase activities of proteasomes are differentially regulated by the major histocompatibility complex-encoded genes for LMP2 and LMP7. *Proceedings of the National Academy of Sciences, USA* **91**, 2913-2917.

Gaffney, D.F., McLauchlan, J., Whitton, J.L. & Clements, J.B. (1985). A modular system for the assay of transcription regulatory signals: the sequnce TAATGARAT is required for herpes simplex virus immediate early gene activation. *Nucleic Acids Research* **13**, 7847-7863.

Galloway, D.A., Fenoglio, C., Shevchuk, M. & McDougall, J.K. (1979). Detection of herpes simplex RNA in human sensory ganglia. *Virology* **95**, 265-268.

Galloway, D.A., Fenoglio, C.M. & McDougall, J.K. (1982). Limited transcription of the herpes simplex virus genome when latent in human sensory ganglia. *Journal of Virology* **41**, 686-691.

Galvin, K., Krishna, S., Ponchel, F., Cimmings, D.E., Carlson, R., Wards, J.R., Isselbacher, K.J., Pillais, S. & Ozturk, M. (1992). The major histocompatibility complex class I antigen-binding protein p88 is the product of the calnexin gene. *Proceedings of the National Academy of Sciences, USA* **89**, 8452-8456.

Gao, M. & Knipe, D.M. (1991). Potential role for herpes simplex virus ICP8 DNA replication protein in stimulation of late gene expression. *Journal of Virology* **65**, 2666-2675.

Garber, D.A., Beverley, S.M. & Coen, D.M. (1993). Demonstration of circularization of herpes simplex virus DNA following infection using pulsed field gel electrophoresis. *Virology* **197**, 459-462.

Garrett, T.P., Saper, M.A., Bjorkman, P.J., Strominger, J.L. & Wiley, D.C. (1989). Specific pockets for the side chains of peptide antigens in HLA-Aw68. *Nature* **342**, 692-696.

Geller, A.I.M. & Breakefield, X.O. (1988). A defective HSV-1 vector expresses E.coli beta-galactosidase in cultured rat peripheral neurons. *Science* **241**, 1667-1669.

Geller, A.I.M & Freese, A. (1990). Infection of cultured central nervous system neurons with a defective herpes simplex virus 1 vector results in stable expression of *Escherichia coli* beta-galactosidase. *Proceedings of the National Academy of Sciences, USA* **87**, 1149-1153.

Geller, A.I.M., Keyomarsi, K., Bryan, J. & Pardee, A.B. (1990). An efficient deletion mutant packaging system for defective herpes simplex virus vectors: Potential applications to human gene therapy and neuronal physiology. *Proceedings of the National Academy of Sciences, USA* **87**, 8950-8954.

Geller, A.I.M (1991). A system using neural cell lines to characterize HSV-1 vectors containing genes which affect neuronal physiology or neuronal promoters. *Journal of Neuroscience Methods* **36**, 91-103.

Geller, A.I.M., During, M.J., Haycock, J.W., Freese, A. & Neve, R.L. (1993). Long-term increases in neurotransmitter release from neuronal cells expressing a constitutively active adenylate cyclase from a HSV-1 vector. *Proceedings of the National Academy of Sciences, USA* **90**, 7603-7607.

Geller, A.I.M., Freese, A., During, M.J. & O'Malley, K.L. (1995). A HSV-1 vector expressing tyrosine hydroxylase causes production and release of L-Dopa from cultured rat striatal cells. *Journal of Neurochemistry* **64**, 487-496.

Gelman, I.H. & Silverstein, S. (1987 a). Herpes simplex virus immediate-early promoters are responsive to virus and cell trans-acting factors. *Journal of Virology* **61**, 2286-2296.

Gelman, I.H. & Silverstein, S. (1987 b). Dissection of immediate-early gene promoters from herpes simplex virus: sequences that respond to the virus transcriptional activators. *Journal of Virology* **61**, 3167-3172.

Gerster, T. & Roeder, R.G. (1988). A herpesvirus trans-activating protein interacts with transcription factor OTF-1 and other cellular proteins. *Proceedings of the National Academy of Sciences, USA* **85**, 6347-6351.

Geschwind, M.D., Kessler, J.A., Geller, A.I. & Federoff, H.J. (1994). Transfer of the nerve growth factor gene into cell lines and cultured neurons using a defective herpes simplex virus vector. *Molecular Brain Research* **24**, 327-335.

Gessner, A., Drjupin, R., Lohler, J., Lother, H. & Lehmann-Grube, F. (1990). IFN-gamma production in tissues of mice during infection with lymphocytic choriomeningitis virus. *Journal of Immunology* **144**, 3160-3165.

Gilbert, M.J., Riddell, S.R., Li, C.R. & Greenberg, P.D. (1993). Selective interference with class I major histocompatibility complex presentation of the major immediate-early protein following infection with human cytomegalovirus. *Journal of Virology* **67**, 3461-3469.

Gilboa, E., Eglitis, M.A., Kantoff, P.W. & Anderson, W.F. (1986). Transfer and expression of cloned genes using retroviral vectors. *Biotechnology* **4**, 504-512.

Gill, M.J., Arlette, J. & Buchan, K. (1988). Herpes simplex virus infection of the hand. *American Journal of Medicine* **84**, 89-93.

Glynne, R., Powis, S.H., Beck, S., Kelly, A., Kerr, L-A. & Trowsdale, J. (1991). A proteasome-related gene between the two ABC transporter loci in the class II region of the human MHC. *Nature* **353**, 357-360.

Goding, J.W. (1976 a). The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. *Journal of Immunological Methods* **10**, 61-66.

Goding, J.W. (1976 b). *Monoclonal antibodies: principles and practice*, pp 87-88, 2nd Ed. London: Academic Press.

Gogate, N., Bakhief, M., Kristensson, K., Norrby, E. & Olsson, T. (1991). Gamma interferon expression and major histocompatibility complex induction during measles and vesicular stomatitis virus infection of the brain. *Journal of Neuroimmunology* **31**, 19-26.

Goins, W.F., Sternberg, L.R., Croen, K.D., Krause, P.R., Hendricks, R.L., Fink, D.J., Straus, S.E., Levine, M. & Glorioso, J.C. (1994). A novel latency-active promoter is contained within the herpes simplex virus type 1 UL flanking repeats. *Journal of Virology* **68**, 2239-2252.

Goldberg, A.L. & Rock, K.L. (1992). Proteolysis, proteasomes and antigen presentation. *Nature* **357**, 375-379.

Gompels, U.A. & Minson, A. (1986). The properties and sequence of glycoprotein H of herpes simplex virus type 1. *Virology* **153**, 230-247.

Goodell, S.E., Quinn, T.C., Mkrtichian, E., Schuffler, M.D., Holmes, K.K. & Corey, L. (1983). Herpes simplex virus proctitis in homosexual men. *New England Journal of Medicine* **308**, 868.

Goodfellow, P.N., Jones, E.A., van Heyningen, V., Soloman, E., Bobrow, M., Miggiano, V. & Bodmer, W.F. (1975). The β_2m gene is on chromosome 15 and not in the HLA region. *Nature* **254**, 267-269.

Goodpasture, E.W. (1929). Herpetic infection, with especial reference to involvement of the nervous system. *Medicine* **8**, 223-243.

Gordon, Y.J., Johnson, B., Romanowski, E. & Araullo-Cruz, T. (1988). RNA complementary to herpes simplex virus type ICP0 gene demonstrated in neurons of human trigeminal ganglia. *Journal of Virology* **62**, 1832-1835.

Goring, D.R., Rossant, J., Clapoff, S., Breitman, L. & Tsui, L.-C. (1987). *In situ* detection of beta-galactosidase in lenses of transgenic mice with a γ-crystallin/LacZ gene. *Science* **235**, 456-458.

Gould, K.G., Cossins, J., Bastin, J., Brownlee, G. & Townsend, A. (1989). A 15 amino acid fragment of influenza nucleoprotein synthesized in the cytoplasm is presented to class I-restricted cytotoxic T lymphocytes. *Journal of Experimental Medicine* **170**, 1051-1056.

Gould, K.G., Scotney, H. & Brownlee, G.G. (1991). Characterization of two distinct major histocompatibility complex class I K^k-restricted T-cell epitopes within the influenza A/PR/8/34 virus haemagglutinin. *Journal of Virology* **65**, 5401-5409.

Gowans, E.J., Jilbert, A.R. & Burrell, C.J. (1989). Detection of specific DNA and RNA sequences in tissues and cells by *in situ* hybridization. In *Nucleic Acid Probes*, pp. 139-158. Edited by Symons, R.H. Boca Raton: CRC Press.

Graham, F.L. & Van der Eb, A.J. (1973). A new technique for the assay of human adenovirus DNA. *Virology* **52**, 456-467.

Gregory, C.D., Murray, R.J., Edwards, C.F. & Rickinson, A.B. (1988). Downregulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus-positive Burkitts lymphoma underlies tumor cell escape from virus-specific T cell surveillance. *Journal of Experimental Medicine* **167**, 1811-1824.

Gresser, I., Tovey, M. G., Maury, C. & Bandu, M. T. (1976). Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis. Newcastle disease and influenza viruses. *Journal of Experimental Medicine* **144**, 1316-1323.

Griffin, A.M. (1991). The nucleotide sequence of the glycoprotein gB gene of infectious laryngotracheitis virus: analysis and evolutionary relationship to the homologous gene from other herpesviruses. *Journal of General Virology* **72**, 393-398.

Gros, P., Croop, J. & Housman, D. (1986). Mammalian multidrug resistance gene: complete cDNA sequence indicates strong homolgy to bacterial transport proteins. *Cell* **47**, 371-380.

Gruenheid, S., Gatzke, L., Meadows, H. & Tufaro, F. (1993). Herpes simplex virus infection and propagation in a mouse L cell mutant lacking heparan sulphate proteoglycans. *Journal of Virology* **67**, 93-100.

Grundy, J.E., McKeating, J.A. & Griffiths, P.D. (1987). Cytomegalovirus strain AD169 binds β2 microglobulin *in vitro* after release from cells. *Journal of General Virology* **68**, 777-784.

Guertin, D.P. & Fan, D.P. (1980). Stimulation of cytolytic T cells by isolated viral peptides HN protein coupled to agarose beads. *Nature* **283**, 308-313.

Guidotti, L.G., Ando, K., Hobbs, M.V., Ishikawa, T., Runkel, L., Schreiber, R.D. & Chisari, F.V. (1994). Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. *Proceedings of the National Academy of Sciences, USA* **91**, 3764-3768.

Haarr, L. & Skulstad, S. (1994). The herpes simplex virus type 1 particle: structure and molecular functions. Review Article. *APMIS* **102**, 321-346.

Hammerling, G.J., Deake, B.D., Mauve, C., Hammerling, U. & McDevitt, H.O. (1974). B lymphocyte alloantigens controlled by the I-region of the major histocompatibility complex in mice. *Immunogenetics* **1**, 68-81.

Hansen, T.H., Myers, N.B. & Lee, D.R. (1988). Studies of two antigenic forms of L^d with disparate β_2-microglobulin (β_2m) association suggests that β_2m facilitates the folding of the α1 and α2 domains during de novo synthesis. *Journal of Immunology* **140**, 3522-3527.

Hanshaw, J.B. & Dudgeon, J.A. (1978). In *Viral diseases of the fetus and newborn*, pp 199, 205, 340. Edited by Saunders. London: Philadelphia.

Harbour, D.A., Hill, T.J. & Blyth, W.A. (1981). Acute and recurrent herpes simplex in several strains of mice. *Journal of General Virology* **55**, 31-40.

Hass, G.M. (1935). Hepato-adrenal necrosis with intranuclear inclusion bodies. *American Journal of Pathology* **11**, 127-142.

Hatherly, L.I., Hayes, K. & Jack, I. (1980). Herpesvirus in an obstetric hospital. Asymptomatic virus excretion in staff members. *The Medical Journal of Australia* **2**, 273-275.

Hayashi, S., Gillam, I.C., Delaney, A.D. & Tener, G.M. (1978). Acetylation of chromosome squashes of *drosophila melanogaster* decreases the background in autoradiographs from hybridization with [^{125}I]-labeled RNA. *The Journal of Histochemistry and Cytochemistry* **26**, 677-679.

Hayashi, K., Kurata, T., Morishima, T. & Nassery, T. (1980). Analysis of the inhibitory effect of peritoneal macrophages on the spread of herpes simplex virus. *Infection and Immunity* **28**, 350-358.

Haynes, R.E., Azimi, P.H. & Cramblett, H. (1968). Fatal herpes virus hominis infections in children. *The Journal of the American Medical Association* **206**, 312-319.

Hayward, G.S., Jacob, R.J., Wadsworth, S.C. & Roizman, B. (1975). Anatomy of herpes simplex virus DNA: evidence for four populations of molecules that differ in the relative orientations of their long and short components. *Proceedings of the National Academy of Sciences, USA* **72**, 4243-4247.

He, X., Treacy, M.N., Simmons, D.M., Ingraham, H.A., Swanson, L.S. & Rosenfeld, M.G. (1989). Expression of a larger family of POU-domain regulatory genes in a mammalian brain development. *Nature* **340**, 35-42.

Heath, W.R., Hurd, M.E., Carbone, F.R. & Sherman, L.A. (1989). Peptide dependent recognition of H-$2K^b$ by alloreactive cytotoxic T lymphocytes. *Nature* **341**, 749-752.

Heath, W.R. & Sherman, L.A. (1990). Cell-type specific recognition of allogeneic cells by alloreactive cytotoxic T cells: A consequence of peptide dependent allorecognition. *European Journal of Immunology* **21**, 153-159.

Heemels, M.T., Schumacher, T.N.M., Wonigeit, K. & Ploegh, H.L. (1993). Peptide translocation by variants of the transporter associated with antigen processing. *Science* **262**, 2059-2063.

Heine, J.W., Honess, R.W., Cassai, E. & Roizman, B. (1974). Proteins specified by herpes simplex virus. XII. The virion polypeptides of type 1 strains. *Journal of Virology* **14**, 640-651.

Heller, M., Dix, R.D., Baringer, J.R., Schachten, J. & Cante, J.E. (1982). Herpetic proctitis and meningitis: recovery of two strains of herpes simplex virus type 1 from cerebrospinal fluid. *Journal of Infectious Diseases* **146**, 584-588.

Henderson, R.A., Michel, H., Sakaguchi, K., Shabanowitz, J., Appella, C., Hunt, D.F. & Engelhard, V.H. (1992). HLA-A2.1-associated peptides in a mutant cell line: a second pathway of antigen presentation. *Science* **255**, 1264-1266.

Hendricks, R.L., Tumpey, T.M. & Finnegan, A. (1992). IFN-gamma and IL-2 are protective in the skin but pathologic in the corneas of HSV-1 infected mice. *Journal of Immunolgy* **149**, 3023-3028.

Hermiston, T.W., Tripp, R.A., Sparer, T., Gooding, L.R. & Wold, W.S.M. (1993). Deletion mutant analysis of the adenovirus type 2 E3-gp 19K protein: identification of sequences within the endoplasmic reticulum luminal domain that are required for class I antigen binding and protection from adenovirus-specific cytotoxic T lymphocytes. *Journal of Virology* **67**, 5289-5298.

Herold, B.C., WuDunn, D., Soltys, N. & Spear, P.G. (1991). Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *Journal of Virology* **65**, 1090-1098.

Herold, B.C., Gerber, S.I., Polonsky, T., Belval, B.J., Shaklee, P.N. & Holme, K. (1995). Identification of structural features of heparin required for inhibition of herpes simplex virus type 1 binding. *Virology* **206**, 1108-1116.

Higgins, C.F., Hyde, S.C., Mimmack, M.M., Gildeadi, V., Gill, D.R. & Gallagher, M.P. (1990). Binding protein-dependent transport systems. *Journal of Bioenergetics and Biomembranes* **22**, 571-592.

Highlander, S.L., Sutherland, S.L., Gage, P.J., Johnson, D.C., Levine, M. & Glorioso, J.C. (1987). Neutralizing monoclonal antibodies specific for herpes simplex virus glycoprotein D inhibit virus penetration. *Journal of Virology* **61**, 3356-3364.

Highlander, S.L., Cai, W., Person, S., Levine, M. & Glorioso, J.C. (1988). Monoclonal antibodies define a domain on herpes simplex virus glycoprotein B involved in virus penetration. *Journal of Virology* **62**, 1881-1888.

Hiles, I.D., Gallagher, M.P., Jamieson, D.J. & Higgins, C.F.C. (1987). Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*. *Journal of Molecular Biology* **195**, 125-142.

Hill, T.J., Field, H.J. & Blyth, W.A. (1975). Acute and recurrent infection with herpes simplex virus in the mouse: a model for studying latency and recurrent disease. *Journal of General Virology* **28**, 341-353.

Hill, T.J., Harbour, D.A. & Blyth, W.A. (1980). Isolation of herpes simplex virus from the skin of clinically normal mice during latent infection. *Journal of General Virology* **47**, 205-207.

Hill, J.M., Sederati, F., Javier, R.T., Wagner, E.K. & Stevens, J.G. (1990). Herpes simplex virus latent phase transcription facilitates *in vivo* reactivation. *Virology* **174**, 117-125.

Hill, A.B., Barnett, B.C., McMichael, A.J. & McGeoch, D.J. (1994). HLA class I molecules are not transported to the cell surface in cells infected with herpes simplex virus type 1 and 2. *Journal of Immunology* **152**, 2736-2741.

Hill, A., Jugovic, P., York, I., Russ, G., Bennik, J., Yewdell, J., Ploegh, H. & Johnson, D. (1995). Herpes simplex virus turns off the TAP to evade host immunity. *Nature* **375**, 411-415.

Ho, D.Y. & Mocarski, E.S. (1988). β-Galactosidase as a marker in the peripheral and neural tissues of the herpes simplex virus-infected mouse. *Virology* **167**, 279-283.

Ho, D.Y. and Mocarski, E.S. (1989). Herpes simplex virus latent RNA (LAT) is not required for latent infection in the mouse. *Proceedings of National Academy of Sciences, USA* **86**, 7596-7600.

Ho, M. (1991). *Cytomegalovirus: Biology and Infection.* Plenum, New York, 2nd edition.

Ho, D.Y., Mocarski, E.S. & Sapolsky, R.M. (1993). Altering central nervous system physiology with a defective herpes simplex virus vector expressing the glucose transporter gene. *Proceedings of the National Academy of Sciences, USA* **90**, 3655-3659.

Hochstenbach, F., David, V., Watkins, S. & Brenner, M.B. (1992). Endoplasmic reticulum resident protein of 90 kilodaltons associates with the T- and B-cell antigen receptors and major histocompatibility complex antigens during their assembly. *Proceedings of the National Academy of Sciences, USA* **89**, 4734-4738.

Hodgman, T.C. & Minson, A.C. (1986). The herpes simplex virus type 2 equivalent of the herpes simplex virus type 1 US7 gene and its flanking sequences. *Virology* **153**, 1-11.

Homan, E.J. & Easterday, B.C. (1980). Isolation of bovine herpes virus-1 from trigeminal ganglia of clinically normal cattle. *American Journal of Veterinary Research* **41**, 309-313.

Homan, E.J. & Easterday, B.C. (1983). Experimental latent and recrudesent bovine herpesvirus-1 infection in calves. *American Journal of Veterinary Research* **44**, 309-313.

Honess, R.W. & Roizman, B. (1973). Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. *Journal of Virology* **12**, 1347-1365.

Honess, R.W. & Roizman, B. (1974). Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *Journal of Virology* **14**, 8-19.

Honess, R.W. & Roizman, B. (1975). Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. *Proceedings of the National Academy of Sciences, USA* **72**, 1276-1280.

Honess, R.W. (1984). Herpes simplex and "the herpes complex": diverse observations and a unifying hypothesis. The eighth Fleming Lecture. *Journal of General Virology* **65**, 2077-2107.

Hood, L., Steinmetz, M. & Malissen, B. (1983). Genes of the major histocompatibility complex of the mouse. *Annual Reviews of Immunolgy* **1**, 529-568.

Hooks, J.J., Burns, W., Hayashi, K., Geis, S. & Notkins, A.L. (1976). Viral spread in the presence of neutralizing antibody: mechanisms of persistence in foamy virus infection. *Infection and Immunity* **14**, 1172-1178.

Horley, K.J., Carpentino, C., Baker, B. & Takei, F. (1989). Molecular cloning of murine intercellular adhesion molecule 1 (ICAM-1). *European Molecular Biological Organization* **8**, 2889-2896.

Horne, R.W. & Wildy, P. (1961). Symmetry in virus architecture. *Virology* **15**, 348-373.

Horohov, D.W., Wyckoff, J.H.III., Moore, R.N. & Rouse, B.T. (1986). Regulation of herpes simplex virus-specific cell-mediated immunity by a specific suppressor factor. *Journal of Virology* **58**, 331-338.

Huang, Q., Vonsattel, J.-P., Schaffer, P.A., Martuza, R.L., Breakefield, X.O. & DiFiglia, M. (1992). Introduction of a foreign gene (*E.coli lacZ*) into rat neostriatal neurons using herpes simplex virus mutants: A light and electronmicroscopic study. *Experimental Neurology* **115**, 303-316.

Huang, Y.Q., Li, J.J., Kaplan, M.H., Poiesz, B., Katabira, E., Zhang, W.C., Feiner, D. & Friedman-Kien, A.E. (1995). Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma patients. *Lancet* **345**, 759-761.

Hunig, T., Tiefenthaler, G., Meyer, Z.U.M., Buschenfelde, K.H. & Meuer, S.C. (1987). Alternative pathway activation of T cells by binding of CD2 to its cell-surface ligand. *Nature* **326**, 298-301.

Hurtley, S.M. & Helenius, A. (1989). Protein oligomerization in the endoplasmic reticulum. *Annual Reviews of Cellular Biology* **5**, 277-307.

Hutchinson, L., Browne, H., Wargent, V., Davis-Poynter, N., Primorac, S., Goldsmith, K., Minson, A.C. & Johnson, D.C. (1992). A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. *Journal of Virology* **66**, 2240-2250.

Igarashi, K., Fawl, R., Roller, R.J. & Roizman, B. (1993). Construction and properties of a recombinant herpes simplex virus 1 lacking both S-component origins of DNA synthesis. *Journal of Virology* **67**, 2123-2132.

Izumi, K.M., McKelvey, A.M., Devi-Rao, G., Wagner, E.K. & Stevens, J.G. (1989). Molecular and biological characterization of a type 1 herpes simplex virus (HSV-1) specifically deleted for expression of the latency-associated transcript (LAT). *Microbial Pathogenesis* **7**, 121-134.

Jackson, M.R. & Peterson, P.A. (1993). Assembly and intracellular transport of MHC I molecules. *Annual Reviews in Cell Biology* **9**, 207-235.

Jackson, M.R., Cohen-Doyle, M.F., Peterson, P.A. & Williams, D.B. (1994). Regulation of MHC class I transport by the molecular chaperone, calnexin (p88, IP90). *Science* **263**, 384-387.

Jacob, R.J. & Roizman, B. (1977). Anatomy of herpes simplex virus DNA. VIII. Properties of the replicating DNA. *Journal of Virology* **23**, 394-411.

Jacob, R.J., Morse, L.S. & Roizman, B. (1979). Anatomy of herpes simplex virus DNA. XII. Accumulation of head-to-tail concatamers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. *Journal of Virology* **29**, 448-457.

Jacobson, J.G., Ruffner, K.L., Kosz-Vnenchak, M., Hwang, C.B.C., Wobbe, K.K., Knipe, D.M. & Coen, D.M. (1993). Herpes simplex virus thymidine kinase and specific stages of latency in murine trigeminal ganglia. *Journal of Virology* **67**, 6903-6908.

Jamieson, A.T. & Subak-Sharpe, J.H. (1974). Biochemical studies on the herpes simplex virus-specified deoxypyrimidine kinase activity. *Journal of General Virology* **24**, 481-492.

Jardetzky, T., Lane, W., Robinson, R.A., Madden, D. & Wiley, D.C. (1991). Identification of self peptides bound to purified HLA-B27. *Nature* **353**, 326-329.

Javier, R.T., Stevens, J.G., Dissette, V.B. & Wagner, E.K. (1988). A herpes simplex virus transcript abundant in latently infected neurons is dispensable for establishment of the latent state. *Virology* **166**, 254-257.

Jefferies, M.A. & Burgert, H-G. (1990). E3/19K from adenovirus 2 is an immunosubversive protein that binds to structural motif regulating the intercellular transport of Major histocompatibility complex class I proteins. *Journal of Experimental Medicine* **172**, 1653-1664.

Jennings, S.R., Rice, P.L., Kloszewski, E.D., Anderson, R.W., Thompson, D.L. & Tevethia, S.S. (1985). Effect of herpes simplex virus types 1 and 2 on surface expression of class I major histocompatibility antigens on infected cells. *Journal of Virology* **56**, 757-766.

Johnson, R.T. (1964). The pathogenesis of herpes virus encephalitis. II. A cellular basis for the development of resistance with age. *Journal of Experimental Medicine* **120**, 359-374.

Johnson, D.C. & Spear, P.G. (1982). Monensin inhibits the processing of herpes simplex glycoproteins, their transport to the cell surface, and the egress of virions from the infected cells. *Journal of Virology* **43**, 1102-1112.

Johnson, D.C., Wittels, M. & Spear, P.G. (1984). Binding to cells of virosomes containing herpes simplex virus type 1 glycoproteins and evidence for fusion. *Journal of Virology* **52**, 238-247.

Johnson, D.C. & Ligas, M.W. (1988). Herpes simplex viruses lacking glycoprotein D are unable to inhibit virus penetration: quantitative evidence for virus-specific cell surface receptors. *Journal of Virology* **62**, 4605-4612.

Johnson, R.M., Lancki, D.W., Fitch, F.W. & Spear, P.G. (1990). Herpes simplex virus glycoprotein D is recognized as antigen by $CD4^+$ and $CD8^+$ T lymphocytes from infected mice. Characterization of T cell clones. *Journal of Immunology* **145**, 702-710.

Joklik, W.K. (1990). Interferons. In *Virology*, pp 393-410, 2nd Ed. Edited by Fields, B.N., Knipe, D.M., et al. New York: Raven Press.

Joly, E., Mucke, L. & Oldstone, M.B.A. (1991). Viral persistence in neurons explained by lack of major histocompatibility class I expression. *Science* **253**, 1283-1285.

Joly, E. & Oldstone, M.B.A. (1992). Neuronal cells are deficient in loading peptides onto MHC class I molecules. *Neuron* **8**, 1185-1190.

Jones, T.R., Hanson, L.K., Sun, L., Slater, J.S., Stenberg, R.M. & Campbell, A.E. (1995). Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *Journal of Virology* **69**, 4380-4841.

Jongeneel, C.V. & Bachenheimer, S.L. (1981). Structure of replicating herpes virus DNA. *Journal of Virology* **39**, 656-660.

Joseph, J., Knobler, R.L., Lublin, F.D. & Hart, M.N. (1989). Differential modulation of MHC class I antigen expression on mouse brain endothelial cells by MHV-4 infection. *Journal of Neuroimmunology* **22**, 241-253.

Joyce, S., Kuzushima, K., Kepecs, G., Angeletti, R.H. & Nathenson, S.G. (1994). Characterization of an incompletely assembled major histocompatiblity class I molecule ($H\text{-}2K^b$) associated with unusually long peptides: Implications for antigen processing and presentation. *Proceedings of the National Academy of Sciences, USA* **91**, 4145-4149.

Kaplitt, M.G., Pfaus, J.G., Kleopoulos, S.P., Hanlon, B.A., Rabkin, S.D. & Pfaff, D.W. (1991). *Molecular and Cellular Neuroscience* **2**, 320-330.

Kaplitt, M.G., Kwong, A.D., Kleopoulus, S.P., Mobbs, C.V., Rabkin, S.D. & Pfaff, D.W. (1994). Preproenkephalin promoter yields region-specific and long-term expression in adult brain after direct *in vivo* transfer via a defective herpes simplex viral vector. *Proceedings of the National Academy of Sciences, USA* **91**, 8979-8983.

Kapoor, A.K., Nash, A.A., Wildy, P., Phelan, J., McLean, C.S. & Field, H.J. (1982 a). Pathogenesis of herpes simplex virus in congenitally athymic mice: the relative roles of cell-mediated and humoral immunity. *Journal of General Virology* **60**, 225-233.

Kapoor, A.K., Nash, A.A. & Wildy, P. (1982 b). Pathogenesis of herpes simplex virus in B-cell suppressed mice: the relative roles of cell-mediated and humoral immunity. *Journal of General Virology* **61**, 127-131.

Kappler, J.W. & Marrack, P.C. (1976). Helper T cells recognize antigen and macrophage surface components simultaneously. *Nature* **262**, 797-799.

Karlin, S., Mocarski, E.S. & Schachtel, G.A. (1994). Molecular evolution of herpesviruses: Genomic and protein sequence comparisons. *Journal of Virology* **68**, 1886-1902.

Karupiah, G., Blanden R.V. & Ramshaw, I.A. (1990). Interferon-γ is involved in the recovery of athymic nude mice from recombinant vaccinia virus/interleukin 2 infection. *Journal of Experimental Medicine* **172**, 1495-1503.

Karupiah, G., Frederickson, T.N., Holmes, K.L., Khairallah, L. & Buller, R.M.L. (1993). Importance of interferons in recovery from mousepox. *Journal of Virology* **67**, 4214-4226.

Katz, J.P., Bodin, E.T. & Coen, D.M. (1990). Quantitative polymerase chain reaction analysis of herpes simplex virus DNA in ganglia of mice infected with replication-incompetent mutants. *Journal of Virology* **64**, 4288-4295.

Kaufman, R.H., Gardner, H.L., Rawls, W.E., Dixon, R.E. & Young, R.L. (1973). Clinical features of herpes genitalis. *Cancer Research* **33**, 1446-1451.

Keane, R.W., Tallent, M.W. & Podack, E.R. (1992). Resistance and susceptibility of neural cells to lysis by cytotoxic lymphocytes and by cytolytic granules. *Transplantation* **54**, 520-526.

Kelly, A., Powis, S.H., Glynne, R., Radley, E., Beck, S. & Trowsdale, J. (1991). Second proteasome-related gene in the human MHC class II region. *Nature* **353**, 667-668.

Kelly, A., Powis, S., Kerr, L., Mockridge, I., Elliott, T., Bastin, J., Uchanska-Ziegler, B., Ziegler, A., Trowsdale, J. & Townsend, A. (1992). Assembly and function of the two ABC transporter proteins encoded in the human major histocompatibility complex. *Nature* **355**, 641-644.

Kemp, L.M., Dent, C.L. & Latchman, D.S. (1990). Octamer motif mediates transcriptional repression of HSV immediate-early genes and octamer-containing cellular promoters in neuronal cells. *Neuron* **4**, 215-222.

Kennedy, P.G.E., Al-Saadi, S.A. & Clements, G.B. (1983). Reactivation of latent herpes simplex virus from dissociated identified dorsal root ganglion cells in culture. *Journal of General Virology* **64**, 1629-1635.

Kieff, E.D., Bachenheimer, S.L. & Roizman, B. (1971). Size, composition and structure of the DNA of subtypes 1 and 2 herpes simplex virus. *Journal of Virology* **8**, 125-129.

Kindt, T. & Singer, D. (1987). Class I major histocompatibility complex genes in vertebrate species: what is the common denominator? *Immunology Research* **6**, 59-65.

Kissonerghis, A-M., Owen, M.J. & Lodish, H.F. (1980). Biosynthesis of HLA-A and HLA-B antigens *in vivo*. *Journal of Biological Chemistry* **255**, 9678-9684.

Klavinskis, L., Geckeler, R. & Oldstone, M.B.A. (1989). Cytotoxic T lymphocyte control of acute lymphocytic choriomeningitis virus infection: interferon- γ, but not tumour necrosis factor-α, displays antiviral activity *in vivo*. *Journal of General Virology* **70**, 3317-3325.

Kleijmeer, M., Kelly, A., Geuze, H., Slot, J. & Trowsdale, J. (1992). MHC-encoded transporters are located in the ER and *cis*-Golgi. *Nature* **357**, 342-344.

Klein, J. (1975). Biology of the mouse histocompatibility complex. New York: Springer-Verlag.

Klein, J., & Hauptfeld, B. (1976). Ia antigens: their serology, molecular relationships, and their role in allograft rections. *Transplantation Reviews* **30**, 83-100.

Klein, J., Juretic, A., Baxevanis, C.N. & Nagy, Z.A. (1981). The traditional and new version of the mouse H-2 complex. *Nature* **291**, 455-460.

Klein, J., Figueroa, F. & David, C.S. (1983). H-2 haplotypes, genes and antigens: second listing. II. The H-2 complex. *Immunogenetics* **17**, 553-596.

Klein, J. (1986). Natural history of the Major Histocompatibility Complex. New York: Wiley Interscience.

Knotts, F.B., Cook, M.L. & Stevens, J.G. (1973). Latent herpes simplex virus in the central nervous system of rabbits and mice. *Journal of Experimental Medicine* **138**, 740-744.

Koelle, D.M., Tigges, M.A., Burke, R.A., Symington, F.W., Riddell, S.R., Abbo, H. & Corey, L. (1993). Herpes simplex virus infection of human fibroblasts and keratinocytes inhibits recognition by cloned $CD8^+$ T lymphocytes. *Journal of Clinical Investigation* **19**, 961-968.

Kohl, S. & Loo, L.S. (1982). Protection of neonatal mice against herpes simplex virus infection. Probable *in vivo* antibody-dependent cellular cytotoxicity. *Journal of Immunolgy* **129**, 370-376.

Kollias, G., Spanopoulou, E., Grosveld, F., Ritter, M., Beech, J. & Morris, R. (1987). Differential regulation of a Thy-1 gene in transgenic mice. *Proceedings of the National Academy of Sciences, USA* **84**, 1492-1496.

Koppelman, B., Zimmerman, D.L., Walter, P. & Brodsky, F.M. (1992). Evidence for peptide transport across microsomal membranes. *Proceedings of the National Academy of Sciences, USA* **89**, 3908-3912.

Kosz-Vnenchak, M., Coen, D.M. & Knipe, D.M. (1990). Restricted expression of herpes simplex virus lytic genes during establishment of latent infection by thymidine kinase-negative mutant viruses. *Journal of Virology* **64**, 5396-5402.

Kramer, M.F. & Coen, D.M. (1995). Quantification of transcripts from the ICP4 and thymidine kinase genes in mouse ganglia latently infected with herpes simplex virus. *Journal of Virology* **69**, 1389-1390.

Krangel, M.S., Orr, H.T. & Strominger, J.L. (1979). Assembly and maturation of HLA-A and HLA-B antigens *in vivo*. *Cell* **18**, 979-991.

Krause, P.R., Croen, K.D., Straus, S.E. & Ostrove, J.M. (1988). Detection and preliminary characterization of herpes simplex virus type 1 transcripts in latently infected human trigeminal ganglia. *Journal of Virology* **62**, 4819-4823.

Krause, P.R., Ostrove, J.M. & Straus, S.E. (1991). The nucleotide sequence, 5' end, promoter domain, and kinetics of expression of the gene encoding the herpes simplex virus type 2 latency-associated transcript. *Journal of Virology* **65**, 5619-5623.

Kristensson, K., Lycke, E. & Sjostrand, J. (1971). Spread of herpes simplex virus in peripheral nerve. *Acta Neuropathologica* **17**, 44-53.

Kristensson, K., Lycke, E., Roytta, M., Svennerholm, B. & Vahlne, A. (1986). Neuritic transport of herpes simplex virus in rat sensory neurons *in vitro*. Effects of substances interacting with microtubular function and axonal flow [Nocodazone, Taxol and erythro-9-3-(2-hydroxynonyl)adenine]. *Journal of General Virology* **67**, 2023-2028.

Kuhlman, P., Moy, V.T., Lollo, B.A. & Brian, A.A. (1991). The accessory function of murine intercellular adhesion molecule-1 in T lymphocyte activation: Contributions of adhesion and co-activation. *Journal of Immunology* **146**, 1773-1782.

Kutish, G., Mainprize, T. & Rock, D. (1990). Characterization of the latency-related transcriptionally active region of the bovine herpesvirus 1 genome. *Journal of Virology* **64**, 5730-5737.

Kvist, S., Ostberg, L., Persson, H., Philipson, L. & Peterson, P.A. (1978). Molecular association between transplantation antigens and a cell surface antigen in an adenovirus-transformed cell line. *Proceedings of the National Academy of Sciences, USA* **75**, 5674-5678.

Kwong, A.D., Kruper, J.A. & Frenkel, N. (1988). Herpes simplex virus virion host shutoff function. *Journal of Virology* **62**, 912-921.

Lagunoff, M. & Roizman, B. (1994). Expression of a herpes simplex virus type 1 open reading frame antisense to the $\gamma_1$34.5 gene and transcribed by an RNA 3' coterminal with the unspliced latency-associated transcript. *Journal of Virology* **68**, 6021-6028.

Lagunoff, M. & Roizman, B. (1995). The regulation of synthesis and properties of the protein product of the open reading frame P of the herpes simplex virus 1 genome. *Journal of Virology* **69**, 3615-3623.

Lakeman, A.D., Nahmias, A.J. & Whitley R.J. (1986). Analysis of DNA from recurrent genital herpes simplex virus isolates by restriction endonuclease digestion. *Sexually Transmitted Diseases* **13**, 61-66.

Lalanne, J.L., Delarbre, C., Gachelin, G. & Kourilsky, P. (1983). A cDNA clone containing the entire coding sequence of a mouse H-2K^d histocompatibility antigen. *Nucleic Acid Research* **11**, 1567-1577.

Lampson, L.A., Fisher, C.A. & Whelan, J.P. (1983). Striking paucity of HLA-A, B, C and β_2-microglobulin on human neuroblastoma cell lines. *Journal of Immunology* **130**, 2471-2478.

Lampson, L.A. & Fisher, C.A. (1984). Weak HLA and β2-microglobulin expression of neuronal cell lines can be modulated by interferon. *Proceedings of the National Academy of Sciences, USA* **81**, 6476-6480.

Larsen, H.S., Russell, R.G. & Rouse, B.T. (1983). Recovery from herpes simplex virus type I infection is mediated by cytotoxic T-lymphocytes. *Infection and Immunity* **41**, 197-204.

Lavi, E., Suzumura, A., Murasko, D.M., Murray, E.M., Silberberg, D.H. & Weiss, S.R. (1988). Tumour necrosis factor induces expression of MHC class I antigens on mouse astrocytes. *Journal of Neuroimmunology* **18**, 245-253.

Lawlor, D.A., Zemmour, J., Ennis, P.D. & Parham, P. (1990). Evolution of class I-MHC genes and proteins: from natural to thymic selection. *Annual Reviews of Immunolgy* **8**, 23-63.

Lawman, M.J.P., Rouse, B.T., Courtney, R.J. & Walker, R.D. (1980). Cell-mediated immunity against herpes simplex induction of cytotoxic T-lymphocytes. *Infection and Immunity* **27**, 133.

Lee, W.C. & Fuller, A.O. (1993). Herpes simplex virus type 1 and pseduorabies virus bind to a common saturable receptor on Vero cells that is not heparan sulphate. *Journal of Virology* **67**, 5088-5097.

Lee, S.P. (1994). Control mechanisms of Epstein-Barr virus persistence. *Seminars in Virology* **5**, 281-288.

Leib, D.A., Bogard, C.L., Kosz-Vnenchak, M., Hicks, K.A., Coen, D.M., Knipe, D.M. & Schaffer, P.A. (1989 a). A deletion mutant of the latency-associated transcript of herpes simplex virus type 1 reactivates from the latent state with reduced frequency. *Journal of Virology* **63**, 2893-2900.

Leib, D.A., Coen, D.M., Bogard, C.L., Hicks, K.A., Yager, D.R., Knipe, D.M., Tyler, K.L. & Schaffer, P.A. (1989 b). Immediate-early regulatory gene mutants define different stages in the establishment and reactivation of herpes simplex virus latency. *Journal of Virology* **63**, 759-768.

Leib, D.A., Nadeau, K.C., Rundle, S.A. & Schaffer, P.A. (1991). The promoter of the latency-associated transcripts of herpes simplex virus type 1 contains a functional cAMP-response element: Role of the latency-associated transcripts and cAMP in reactivation of viral latency. *Proceedings of the National Academy of Sciences, USA* **88**, 48-52.

Leib, D.A. & Olivo, P.D. (1993). Gene delivery to neurons: Is herpes simplex virus the right tool for the job? *Bioessays* **15**, 547-554.

Leist, T.P., Sandri-Goldin, R.M. & Stevens, J.G. (1989 a). Latent infections in spinal ganglia with thymidine kinase-deficient herpes simplex virus. *Journal of Virology* **63**, 4976-4978.

Leist, T.P., Eppler, M. & Zinkernagel, R.M. (1989 b). Enhanced virus replication and inhibition of lymphocytic choriomeningitis virus disease in anti-gamma interferon treated-mice. *Journal of Virology* **63**, 2813-2819.

Leung, K.N., Nash, A.A., Sia, D.Y. & Wildy, P. (1984). Clonal analysis of T cell responses to herpes simplex virus: Isolation, characterization and antiviral properties of an antigen-specific helper T cell clone. *Immunology* **53**, 623-633.

Levy, F., Gabathuler, R., Larrson, R. & Kvist, S. (1991). ATP is required for *in vitro* assembly of MHC class I antigens but not for transfer of peptides across the ER membrane. *Cell* **67**, 265-274.

Ligas, M.W. & Johnson, D.C. (1988). A herpes simplex virus mutant in which glycoprotein D sequences are replaced by β-galactosidase sequences binds to but is unable to penetrate into cells. *Journal of Virology* **62**, 1486-1494.

Lillycrop, K.A., Dent, C.L., Wheatley, S.C., Beech, M.N., Ninkina, N.N., Wood, J.N. & Latchman, D.S. (1991). The octamer-binding protein Oct-2 represses HSV immediate-early genes in cell lines derived from latently infectable sensory neurons. *Neuron* **7**, 381-390.

Lillycrop, K.A., Estridge, J.K. & Latchman, D.S. (1993). The octamer binding protein OCT-2 inhibits transactivation of the herpes simplex virus immediate-early genes by the virion protein Vmw65. *Virology* **196**, 888-891.

Lillycrop, K.A., Howard, M.K., Estridge, J.K. & Latchman, D.S. (1994). Inhibition of herpes simplex virus infection by ectopic expression of neuronal splice variants of the Oct-2 transcription factor. *Nucleic Acids Research* **22**, 815-820.

Lindsley, M.D., Thiemann, F.L. & Rodriguez, M. (1988). Expression of class I antigen in C57/BL10 and SJL mice following Theilers virus infection. *Journal of Neuropathology and Experimental Neurology* **47**, 324-334.

Little, S.P., Jofre, J.T., Courtney, R.J. & Schaffer, P.A. (1981). A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. *Virology* **115**, 149-160.

Liu, Y., King, N., Kesson, A., Blanden, R.V. & Mullbacher, A. (1989). Flavivirus infection up-regulates the expression of class I and class II major histocompatibilty antigens on and enhances T cell recognition of astrocytes *in vitro*. *Journal of Neuroimmunology* **21**, 157-168.

Lodmell, D.L., Niwa, A., Hayashi, K. & Notkins, A.L. (1973). Prevention of cell-to-cell spread of herpes simplex virus by leukocytes. *Journal of Experimental Medicine* **137**, 706-720.

Lofgren, K.W., Stevens, J.G., Marsden, H.W. & Subak-Sharpe, J.H. (1977). Temperature-sensitive mutants of herpes simplex virus differ in the capacity to establish latent infection in mice. *Virology* **76**, 440-443.

Lokensgard, J.R., Thawley, D.G. & Molitor, T.W. (1990). Pseudorabies virus latency: restricted transcription. *Archives of Virology* **110**, 129-136

Longnecker, R., Roizman, B. & Meignier, B. (1988). Herpes simplex viruses as vectors: properties of prototype vaccine strain suitable for use as a vector. In *Viral Vectors*. Edited by Gluzman, Y. & Hughes, S.H. New York: Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Lonsdale, D.M., Brown, S.M., Subak-Sharpe, J.H., Warren, K.G. & Koprowski, H. (1979). The polypeptide and the DNA restriction enzyme profiles of spontaneous isolates of herpes simplex virus type 1 from explants of human trigeminal, superior cervical and vagus ganglia. *Journal of General Virology* **43**, 151-171.

Lopez, C., Ryshke, R. & Bennett, M. (1980). Marrow dependent cells depleted by ^{89}Sr mediate genetic resistance to herpes simplex virus type 1 infections in mice. *Infection and Immunity* **28**, 1028-1032.

Lopez, C. (1985). Natural resistance mechanisms in herpes simplex virus infection. In: *The Herpesviruses* pp 37-68 vol.4. Edited by Roizman, B. & Lopez, C. New York: Plenum Press.

Lozzio, B.B. & Lozzio, C.B. (1979). Properties and usefulness of the original K-562 human myelogenous leukemia cell line. *Leukemia Research* **3**, 363-370.

Lucin, P., Pavic, I., Jonjic, S. & Koszinowski, U.H. (1992). Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. *Journal of Virology* **66**, 1977-1984.

Mackem, S. & Roizman, B. (1982). Regulation of α genes of herpes simplex virus: the α 27 gene promoter-thymidine kinase chimera is positively regulated in converted L cells. *Journal of Virology* **43**, 1015-1023.

Madden, D.R., Gorga, J.C., Strominger, J.L. & Wiley, D.C. (1991). The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature* **353**, 321-325.

Maehlen, J., Nennesmo, I., Olsson, A-B., Olsson, T., Daa Schroder, H. & Kristensson, K. (1989). Peripheral nerve injury causes transient expression of MHC class I antigens in rat motor neurons and skeletal muscles. *Brain Research* **481**, 368-372.

Maggioncalda, J., Mehta, A., Fraser, N.W. & Block, T.M. (1994). Analysis of a herpes simplex virus type 1 LAT mutant with a deletion between the putative promoter and the 5' end of the 2.0-kilobase transcript. *Journal of Virology* **68**, 7816-7824.

Main, E.K., Monos, D.S. & Lampson, L.A. (1988). IFN-treated neuroblastoma cell lines remain resistant to T cell-mediated allokilling, and susceptible to non-MHC-restricted cytotoxicity. *Journal of Immunolgy* **141**, 2943-2950.

Makagoba, M.W., Sanders, M.E., Luce, G.E., Gugel, E.A., Dustin, M.L., Springer, T.A. & Shaw, S. (1988). Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1-dependent adhesion in T cell-mediated cytotoxicity. *European Journal of Immunology* **18**, 637-640.

Malarkannan, S., Goth, S., Buchholz, D.R. & Shastri, N. (1995). The role of MHC class I molecules in the generation of endogenous peptide/MHC complexes. *Journal of Immunology* **154**, 585-598.

Mandel, G., Cooperman, S.S., Maue, R.A., Goodman, R.H. & Brehm, P. (1988). Selective induction of brain type II Na channels by nerve growth factor. *Proceedings of the National Academy of Sciences, USA* **85**, 924-928.

Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Manservigi, R., Spear, P.G. & Buchan, A. (1977). A cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. *Proceedings of the National Academy of Sciences, USA* **74**, 3913-3917.

Maples, J.A. (1985). A method for the covalent attachment of cells to glass slides for use in immunohistochemistry assays. *American Journal of Clinical Pathology* **83**, 356-363.

Margolese, L., Waneck, G.L., Suzuki, C.K., Degen, E., Flavell, R.A. & Williams, D.B. (1993). Identification of the region on the major histocompatibility molecule that interacts with the molecular chaperone, p88 (calnexin, IP90). *Journal of Biological Chemistry* **268**, 17959-17966.

Marozzi, A., Meneveri, R., Bunone, G., De Santis, C., Lapolco, L., Beretta, A., Agresti, A., Siccardi, A.G., Della Valle, G. & Ginelli, E. (1993). Expression of β_2m-free HLA class I heavy chains in neuroblastoma cell lines. *Scandanavian Journal of Immunology* **37**, 661-667.

Marrack, P. & Kappler, J. (1986). The antigen-specific major histocompatibility complex-restricted receptor on T cells. *Advances in Immunology* **38**, 1-30.

Marrack, P. & Kappler, J. (1987). The T cell receptor. *Science* **238**, 1073-1079.

Marsden, H.S., Campbell, M.E.M., Haarr, L., Frame, M.C., Parris, D.S., Murphy, M., Hope, R.G., Muller, M.T. & Preston, C.M. (1987). The 65,000-M_r DNA-binding and virion *trans*-inducing proteins of herpes simplex virus type 1. *Journal of Virology* **61**, 2428-2437.

Martin, R.G., Dawson, C.R., Jones, P., Togni, B., Lyons, C. & Oh, J.O. (1977). Herpes virus in sensory and autonomic ganglia after eye infection. *Archives of Ophthalmology* **95**, 2053-2056.

Martin, S., Courtney, R.J., Fowler, G. & Rouse, B.T. (1988). Herpes simplex virus type 1 specific cytotoxic T lymphocytes recognize virus nonstructural proteins. *Journal of Virology* **62**, 2265-2273.

Martinez, C.K. & Monaco, J.J. (1991). Homology of the proteasome subunits to a major histocompatibility complex-linked LMP gene. *Nature* **353**, 664-667.

Martz, E. (1993). Overview of CTL-target adhesion and other critical events in the cytotoxic mechanism. In: *Cytotoxic Cells: Recognition, Effector Function, Generation, and Methods*, pp 9-45. Edited by Sitkovsky, M.V. & Henkart, P.A. Boston: Birkhauser.

Massa, P.T., Dorries, R. & terMeulen, V. (1986). Virus particles induce Ia antigen expression on astrocytes. *Nature* **320**, 543-546.

Massa, P.T., Ozata, K. & McFarlin, D.E. (1993). Cell type-specific regulation of major histocompatibilty complex (MHC) class I gene expression in astrocytes, oligodendrocytes and neurons. *Glia* **8**, 201-207.

Masucci, M.G., Torsteinsdottir, S., Colombani, J., Brautbar, C., Klein, E. & Klein, G. (1987). Down-regulation of class I HLA antigens and of the Epstein-Barr virus-encoded latent membrane protein in Burkitt lymphoma lines. *Proceedings of the National Academy of Sciences, USA* **84**, 4567-4571.

Masucci, M.G., Stam, N.J., Torsteinsdottir, S., Neefjes, JJ., Klein, G. & Ploegh, H.L. (1989). Allele-specific down-regulation of MHC class I antigens in Burkitt lymphoma lines. *Cellular Immunology* **120**, 396-400.

Masucci, M.G., Zhang, Q-J., Gavioli, R., DeCampos-Lima, P.O., Murray, R.J., Brooks, J., Griffin, H., Ploegh, H.L. & Rickinson, A.B. (1992). Immune escape by Epstein-Barr virus (EBV) carrying Burkitt's lymphoma : *in vitro* reconstitution of sensitivity to EBV-specific cytotoxic T cells. *International Immunology* **4**, 1283-1292.

Matsui, K., Boniface, J.J., Reay, P.A., Schild, H., Fazekas De St. Groth, B. & Davis, M.M. (1991). Low affinity interaction of peptide-MHC complexes with T cell receptors. *Science* **254**, 1788-1791.

Matsumura, M., Fremont, D., Peterson, P.A. & Wilson I.A. (1992) Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science* **257**, 927-934.

Maue, R.A., Kraner, S.D., Goodman, R.H. & Mandel, G. (1990). Neuron-specific expression of the rat brain type II sodium channel gene is directed by upstream regulatory elements. *Neuron* **4**, 223-231.

Mauerhoff, T., Pujol-Borrell, R., Mirakian, R. & Bottazzo, G.F. (1988). Differential expression and regulation of major histocompatibility complex (MHC) products in neural and glial cells of the human fetal brain. *Journal of Neuroimmunology* **8**, 271-289.

Mavromara-Nazos, P., Ackermann, M. & Roizman, B. (1986). Construction and properties of a viable herpes simplex virus recombinant lacking coding sequences of the α47 gene. *Journal of Virology* **60**, 807-812.

Mavromara-Nazos, P. & Roizman, B. (1987). Activation of herpes simplex virus γ_2 genes by viral DNA replication. *Virology* **161**, 593-598.

McCarthy, A.M., McMahan, L. & Schaffer, P.A. (1989). Herpes simplex virus type 1 ICP27 deletion mutant exhibit altered patterns of transcription and are DNA deficient. *Journal of Virology* **63**, 18-27.

McClain, D.S. & Fuller, A.O. (1994). Cell-specific kinetics and efficiency of herpes simplex type 1 entry and determined by two distinct phases of attachment. *Virology* **198**, 690-702.

McDevitt, H.O., Deak, B.D., Shreffler, D.C., Klein, J., Stimpfling, J.H. & Snell, G.D. (1972). Genetic control of the immune response. Mapping of the Ir.1 locus. *Journal of Experimental Medicine* **135**, 1259-1278.

McDevitt, H.O., Delovitch, T.L., Press, J.J. & Murphy, D.B. (1976). Genetic and functional analysis of the Ia antigens: their possible role in regulating the immune response. *Transplantation Reviews* **30**, 197-235.

McGeoch, D.J., Moss, H.W.M., McNab, D. & Frame, M.C. (1987). DNA sequence and genetic content of the Hind III 1 region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glcoprotein G, and evolutionary comparisons. *Journal of General Virology* **68**, 19-38.

McGeoch, D.J., Dalrympic, M.A., Davison, A.I., Dolan, A., Frame, M.C., McNab, D., Perry, I.J., Scott, H. & Lavloi, P. (1988). The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. *Journal of General Virology* **69**, 1531-1571.

McGeoch, D.J. (1989). The genomes of human herpesviruses: contents, relationships and evolution. *Annual Reviews in Microbiology* **43**, 235-265.

McGeoch, D.J. (1990). Evolutionary relationships of virion glycoprotein genes in the S regions of alphaherpesvirus genomes. *Journal of General Virology* **71**, 2361-2367.

McGeoch, D.J., Cunningham, C., McIntyre, G. & Dolan, A. (1991). Comparative sequence analysis of the long repeat regions and adjoining parts of the long unique regions in the genomes of herpes simplex viruses type 1 and 2. *Journal of General Virology* **72**, 3057-3075.

McGeoch, D.J. (1992). Molecular evolution of large DNA viruses of eukaryotes. *Seminars in Virology* 3, 399-409.

McGeoch, D.J., Barnett, B.C. & MacLean, C.A. (1993). Emerging functions of alphaherpesvirus genes. *Seminars in Virology* **4**, 125-134.

McGrath, J.P. & Varshavsky, A. (1989). The yeast STE6 gene encodes a homologue of the mammalian multidrug resistance g-glycoprotein. *Nature* **340**, 400-404.

McKnight, J.L., Kristie, T.M. & Roizman, B. (1987). Binding of the virion protein mediating α gene induction in herpes simplex virus 1-infected cells to its *cis* site requires cellular proteins. *Proceedings of the National Academy of Sciences, USA* **84**, 7061-7065.

McLean, I.W. & Nakane, P.K. (1974). Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. *The Journal of Histochemistry and Cytochemistry* **22**, 1077-1083.

McLennan, J.L. & Darby, G. (1980). Herpes simplex virus latency: the cellular location of virus in dorsal root ganglia and the fate of the infected cell following virus activation. *Journal of General Virology* **51**, 233-243.

Meignier, B., Longnecker, R., Mavromara-Nazos, P., Sears, A.E. & Roizman, B. (1988). Virulence and establishment of latency by genetically engineered deletion mutants of herpes simplex virus. *Virology* **162**, 251-254.

Mellencamp, M.W., O'Brien, P.C.M. & Stevenson, J.R. (1991). Pseudorabies virus-induced suppression of major histocompatibility complex class I antigen expression. *Journal of Virology* **65**, 3365-3368.

Mellerick, D.M. & Fraser, N.W. (1987). Physical state of the latent herpes simplex virus genome in a mouse model system: evidence suggesting an episomal state. *Virology* **158**, 265-275.

Mellor, A.L., Weiss, E.H., Kress, M., Jay, G. & Flavell, R.A. (1984). A nonpolymorphic class I gene in the murine major histocompatibility complex. *Cell* **36**, 139-144.

Menco, B.P.M. (1986). A survey of ultrarapid cryofixation methods with particular emphasis on applications to freeze fracturing, freeze etching and freeze substitution. *Journal of Electron Microscopy* **4**, 177-240.

Merigan, T.C. & Stevens, D.A. (1971). Viral infections in man associated with acquired immunological deficiency states. *Federation Proceedings* **30**, 1858-1864.

Mertz, G.J., Benedetti, J., Ashley, R., Selke, S.A. & Corey, L. (1992). Risk factors for the sexual transmission of genital herpes. *Annals of Internal Medicine* **116**, 197-202.

Michael, N., Spector, D., Mavromara-Nazos, P., Kristie, T.M. & Roizman, B. (1988). The DNA binding properties of the major regulatory protein $\alpha 4$ of herpes simplex viruses. *Science* **239**, 1531-1534.

Michaelson, J., Rothenberg, E. & Boyse, E.A. (1980). Genetic polymorphism of murine β_2 microglobulin detected biochemically. *Immunogenetics* **11**, 93-95.

Michaelson, J. (1981). Genetic polymorphism of β_2 microglobulin (β_2m) maps to the H-3 region of chromosome 2. *Immunogenetics* **13**, 167-171.

Miller, G. (1990). Epstein-Barr virus. In *Virology*, pp 1921-1958, 2nd Ed., Edited by Fields, B.N., Knipe, M., *et al.* New York: Raven Press.

Miller, D.G., Adam, M.A. & Miller, D.A. (1990). Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. *Molecular and Cellular Biology* **10**, 4239-4242.

Mitchell, W.J., Deshmane, S.L., Dolan, A., McGeoch, D.J. & Fraser, N.W. (1990 a). Characterization of herpes simplex type 2 transcription during latent infection of mouse trigeminal ganglia. *Journal of Virology* **64**, 5342-5348.

Mitchell, W.J., Lirette, R.P. & Fraser, N.W. (1990 b). Mapping of low abundance latency-associated RNA in the trigeminal ganglia of mice latently infected with herpes simplex virus type 1. *Journal of General Virology* **71**, 125-132.

Miyazaki, J-I., Appella, E. & Ozato, K. (1986). Intracellular transport blockage caused by disruption of the disulphide bridge in the third external domain of a major histocompatibility complex class I antigen. *Proceedings of the National Academy of Sciences, USA* **83**, 757-761.

Mocarski, E.S., Post, L.E. & Roizman, B. (1980). Molecular engineering of the herpes simplex virus genome: insertion of a second L-S junction into the genome causes additional genome inversions. *Cell* **22**, 243-255.

Mocarski, E.S. & Roizman, B. (1982 a). Herpesvirus-dependent amplification and inversion of a cell-associated viral thymidine kinase gene flanked by viral *a* sequence and linked to an origin of viral DNA replication. *Proceedings of the National Academy of Sciences, USA* **79**, 5626-5630.

Mocarski, E.S. & Roizman, B. (1982 b). The structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. *Cell* **31**, 89-97.

Moingeon, P., Chang, H.C., Sayre, P.H., Clayton, L.K., Alcover, A., *et al.* 1989. The structural biology of CD2. *Immunology Reviews* **111**, 111-144.

Momburg, F., Koch, N., Moller, P., Moldenhaver, G. & Hammerling, G.J. (1986) In vivo induction of H-2K/D antigens by recombinant interferon-γ. *European Journal of Immunology* **16**, 551-554.

Momburg, F., Oritz-Navarette, V., Neefjes, J., Goulmy, E., van der Wal, Y., Spits, H., Powis, S.J., Butcher, G.W., Howard, J., Walden, P. & Hammerling, G.J. (1992). Proteasome subunits encoded by the major histocompatibility complex are not essential for antigen presentation. *Nature* **360**, 174-177.

Momburg, F., Roelse, J., Howard, J.C., Butcher, G.W., Hammerling, G.J. & Neefjes, J.J. (1994 a). Selectivity of MHC-encoded peptide transporters from the human, mouse and rat. *Nature* **367**, 628-651.

Momburg, F., Roelse, J., Hammerling, G.J. & Neefjes, J.J. (1994 b). Peptide size selection by major histocomatibility complex-encoded peptide transporter. *Journal of Experimental Medicine* **179**, 1613-1623.

Monaco, J.J., Cho, S. & Attaya, M. (1990). Transport protein genes in the murine MHC: possible implications for antigen processing. *Science* **250**, 1723-1726.

Monaco, J.J. (1992). Genes in the MHC that may affect antigen processing. *Current Opinion in Immunology* **4**, 70-73.

Moore, K.W., Sher, B.T., Sun, Y.H., Eakle, K.A. & Hood, L. (1982). DNA sequence of a gene encoding a BALB/c mouse L^d transplantation antigen. *Science* **215**, 679-682.

Moore, M.W., Carbone, F.R. & Bevan, M.J. (1988). Introduction of soluble protein into the class I pathway of antigen processing and presentation. *Cell* **54**, 777-785.

Morahan, P.S. & Morse, S.S. (1979). Macrophage-virus interactions. In: *Virus-lymphocyte interactions: implications for disease,* pp 17-35. Edited by Proffitt, M. New York: Elsevier.

Morahan, P.S., Morse, S.S. & McGeorge, M.B. (1980). Macrophage extrinsic antiviral activity during herpes simplex virus infection. *Journal of General Virology* **46**, 291-300.

Morahan, P.S., Thomson, T.A., Kohl, S. & Murray, B.K. (1981). Immune responses to labial infection of BALB/c mice with herpes simplex virus type 1. *Infection and Immunity* **32**, 180-187.

Morahan, P.S., Dempsy, W.L., Volkman, A. & Connor, J. (1986). Antimicrobial activity of various immuno-modulators: independence from normal levels of circulating monocytes and natural killer cells. *Infection and Immunity* **51**, 87-93.

Morello, D., Daniel, F., Baldacci, P., Cayre, Y., Gachelin, G. & Kourilisky, P. (1982). Absence of significant H-2 and β_2-microglobulin mRNA by mouse embryonal carcinoma cells. *Nature* **296**, 260-262.

Morgan, C., Holden, M. & Jones, E.P. (1959). Electron microscopic observations on the development of herpes simplex virus. *Journal of Experimental Medicine* 110, 643-656.

Morgan, C., Rose, H.M. & Mednis, B. (1968). Electron microscopy of herpes simplex virus. 1. Entry. *Journal of Virology* **2**, 507-516.

Mori, R., Tasaki, T., Kimura, G. & Takeya, K. (1967). Depression of acquired resistance against herpes simplex virus infections in neonatally thymectomized mice. *Arch. Gesamte Virusforsch* **21**, 459-463.

Moriarty, G.C., Moriarty, C.M. & Sternberger, L.A. (1973). Ultrastructural immunocytochemistry by unlabelled antibodies and the peroxidase-antiperoxidase complex (PAP). A technique more sensitive than radioimmunoassay. *Journal of Histochemistry and Cytochemistry* **21**: 825-833.

Nagafuchi, S., Oda, H., Mori, R. & Taniguchi, T. (1979). Mechanisms of acquired resistance against herpes simplex virus infections as studied in nude mice. *Journal of General Virology* **44**, 715-723.

Nagafuchi, S., Hayashida, I., Higa, K., Wada, T. & Mori, R. (1982). Role of Lyt-2 positive immune cells in recovery from herpes simplex virus infection in mice. *Microbiology and Immunology* **26**, 359-362.

Nahmias, A.J., Josey, W.E. & Naib, Z.M. (1967). Neonatal herpes simplex infection. *The Journal of the American Medical Association* **199**, 164-168.

Nahmias, A.J., Josey, W.E., Naib, Z.M., Luce, C. & Duffey, C. (1970 a). Antibodies to herpesvirus hominis type 1 and 2 in humans. 1. Patients with genital herpetic infections. *American Journal of Epidemiology* **91**, 539-546.

Nahmias, A.J., Alford, C. & Korones, S. (1970 b). Infection of the newborn with herpes virus hominis. *Advances in Pediatrics* **17**, 185-226.

Nahmias, A.J. & Roizman, B. (1973). Infection with herpes simplex viruses 1 and 2. *The New England Journal of Medicine* **289**, 667-674,719-725,781-789.

Nahmias, A.J., Keyserling, H. & Lee, F.K. (1989). Herpes simplex viruses 1 and 2. In *Viral Infections of Humans, Epidemiology and Control*, pp. 393-417, 3rd Ed. Edited by Evans, A.S. New York: Plenum.

Nash, A.A., Field, H.J. & Quartey-Papafio, R. (1980 a). A Cell-mediated Immunity in HSV-infected Mice: Induction, Characterization and Anti-viral Effects of Delayed Type Hypersensitivity. *Journal of General Virology* **48**, 351-357.

Nash, A.A., Quartey-Papafio, R. & Wildy, P. (1980 b). Cell-mediated immunity in herpes simplex virus-infected mice: functional analysis of lymph node cells during periods of acute and latent infection, with reference to cytotoxic and memory cells. *Journal of General Virology* **49**, 309-317.

Nash, A.A., Phelan, J. & Wildy, P. (1981). Cell-mediated immunity in herpes simplex virus-infected mice: H-2 mapping of delayed type I infection is mediated by cytotoxic T-lymphocytes. *Journal of Immunology* **126**, 1260-1262.

Nash, A.A. & Gell, P.G.H. (1983). Membrane phenotype of murine effector and suppressor T cells involved in delayed type hypersensitivity and protective immunity to herpes simplex virus. *Cellular Immunology* **75**, 343-355.

Nash, A.A., Jayasuriya, A., Phelan, J., Cobbold, S.P., Waldmann, H. & Prospero, T. (1987). Different roles for L3T4+ and Lyt2+ T cell subsets in the control of an acute herpes simplex virus infection of the skin and nervous system. *Journal of General Virology* **68**, 825-833.

Nash, A.A. & Cambouropoulus, P. (1993). The immune response to herpes simplex virus. *Seminars in Virology* **4**, 181-186.

Neefjes, J.J., Smit, L., Gehrmann, M. & Ploegh, H.L. (1992). The fate of three subunits of major histocompatibility complex class I molecules. *European Journal of Immunology* **22**, 1609-1614.

Neefjes, J.J., Momburg, F. & Hammerling, G.H. (1993). Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter. *Science* 261, 769-771.

Neumann, H., Cavalie, A., Jenne, D.E. & Wekerle, H. (1995). Induction of MHC class I genes in neurons. *Science* **269**, 549-552.

Newcomb, W.W. & Brown, J.C. (1991). Structure of the herpes simplex virus capsid: effects of extraction with guanidine hydrochloride and partial reconstitution of extracted capsids. *Journal of Virology* **65**, 613-620.

Nguyen, L., Knipe, D.M. & Finberg, R.W. (1994). Mechanism of virus-induced Ig subclass shifts. *Journal of Immunology* **152**, 478-484.

Niedermann, G., Butz, S., Ihlenfeldt, H.G., Grimm, K., Lucchian, M., Hoschutzky, H., Jung, G., Maier, B. & Eichmann, K. (1995). Contribution of proteasome-mediated proteolysis to the heirarchy of epitopes presented by major histocompatibility complex class I molecules. *Immunity* **2**, 289-299.

Nii, S., Morgan, C., Rose, H.M. & Hsu, K.C. (1968). Electron microscopy of herpes simplex virus. IV. Studies with ferritin conjugated antibodies. *Journal of Virology* **2**, 1172-1184.

Nishioka, Y. & Silverstein, S. (1977). Degradation of cellular mRNA during infection by herpes simplex virus. *Proceedings of the National Academy of Sciences, USA* **74**, 2370-2374.

Nishioka, Y. & Silverstein, S. (1978). Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukemia cells infected with herpes simplex virus type 1. *Journal of Virology* **25**, 422-426.

Noda, M., Furutini, Y., Takahashi, H., Toysato, M., Tanabe, T., Shimizu, S., Kikyotani, S., Kayano, T., Hirose, T., Inayama, S. & Numa, S. (1983). Cloning and sequence analysis of calf cDNA and human genomic DNA encoding alpha-subunit precursor of muscle acetylcholine receptor subunits. *Nature* **302**, 818-823.

O'Brien, W.J. & Taylor, J.L. (1989). The isolation of herpes simplex virus from rabbit corneas during latency. *Investigative Ophthalmology and Visual Science* **30**, 357-362.

O'Connor, C.G. & Ashman, L.K. (1982). Application of nitrocellulose transfer technique and alkaline phosphatase conjugated anti-immunoglobulin for determination of the specificity of monoclonal antibodies to protein mixtures. *Journal of Immunological Methods* **54**, 267-271.

O'Hare, P. & Goding, C.R. (1988). Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind a common factor and are both targets for virion transactivation. *Cell* **52**, 435-445.

O'Hare, P., Goding, C.R. & Haigh, A. (1988). Direct combinatorial interaction between a herpes simplex virus regulatory protein and a cellular octamer-binding factor mediates specific induction of virus immediate-early gene expression. *European Molecular Biological Organization* **7**, 4231-4238.

O'Rourke, A.M., Apgar, J.R., Kane, K.P., Martz, E. & Mescher, M.F. (1991). Cytoskeletal function in CD8- and T cell receptor-mediated interaction of cytotoxic T lymphocytes with class I protein. *Journal of Experimental Medicine* **173**, 241-249.

O'Rourke, A.M. & Mescher, M.F. (1993). The roles of CD8 in cytotoxic T lymphocyte function. *Immunology Today* **14**: 183-188.

Oakes, J. E. (1975). Role of cell-mediated immunity in the resistance of mice to subcutaneous herpes simplex infection. *Infection and Immunity* **12**, 166-172.

Olson, L.C., Buescher, E.C., Artenstein, M.S. & Parkman, P.D. (1967). Herpesvirus infections of the human central nervous system. *The New England Journal of Medicine* **277**, 1271-1277.

Openshaw, H. (1983). Latency of herpes simplex virus in ocular tissue of mice. *Infection and Immunity* **39**, 960-962.

Oritz-Navarrete, V. & Hammerling, G.J. (1991). Surface appearance and instability of empty H-2 class I molecules under physiological conditions. *Proceedings of the National Academy of Sciences USA* **88**, 3549-3597.

Oritz-Navarrete, V., Seelig, V.A., Gernold, M., Frentzel, S., Kloetzel, P.M. & Hammerling, G.J. (1991). Subunit of the '20S' proteasome (multicatalytic proteinase) encoded by the major histocompatibility complex. *Nature* **353**, 662-664.

Orlowski, M. (1990). The multicatalytic proteinase complex, a major extrachromosomal proteolytic system. *Biochemistry* **29**, 10289-10297.

Ortmann, B., Androlewicz, M.J. & Cresswell, P. (1994). MHC class I/β2-microglobulin complexes associate with TAP transporters before peptide binding. *Nature* **368**, 864-867.

Ou, W.-J., Cameron, P.H., Thomas, D.Y. & Bergeron, J.J.M. (1993). Association of folding intermediates of glycoproteins with calnexin during protein maturation. *Nature* **364**, 771-776.

Owen, M.J., Kissonerghis, A.M. & Lodish, H.L. (1980). Biosynthesis of HLA-A and HLA-B antigens *in vivo*. *Journal of Biological Chemistry* **255**, 9628-9684.

Paabo, S., Nilsson, T. & Peterson, P.A. (1986). Adenoviruses of subgenera B, C, D and E modulate cell-surface expression of major histocompatibility complex class I antigens. *Proceedings of the National Academy of Sciences, USA* **83**, 9665-9669.

Paabo, S., Bhat, B.M., Wold, W.S.M. & Peterson, P.A. (1987). A short sequence in the COOOH-terminus makes an adenovirus membrane glycoprotein a resident of the endoplasmic reticulum. *Cell* **50**, 311-317.

Palella, T.D., Silverman, L.J., Schroll, C.T., Homa, F.L., Levine, M. & Kelly, W.N. (1988). Herpes simplex virus-mediated human hypoxanthine-guanine phosphoribosyltransferase gene transfer into neuronal cells. *Molecular and Cellular Biology* **8**, 457-460.

Palella, T.D., Hidaka, Y., Silverman, L.J., Levine, M., Glorioso, J. & Kelley, W.N. (1989). Expression of human HPRT mRNA in brains of mice infected with a recombinant herpes simplex virus-1 vector. *Gene* **80**, 137-144.

Palmiter, R.D. & Brinster, R. (1985). Transgenic mice. *Cell* **41**, 343-345.

Palmiter, R.D., Chen, H.Y., Messing, A. & Brinster, R.L. (1985). SV40 enhancer and large-T antigen are instrumental in development of choroid plexus tumours in transgenic mice. *Nature* **316**, 457-460.

Palmiter, R.D., Behringer, R.P., Quaife, C.J., Maxwell, F., Maxwell, I.H. & Brinster, R. (1987). Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. *Cell* **50**, 435-443.

Pamer, E.G., Harty, J.T. & Bevan, M.J. (1991). Precise prediction of a dominant class I MHC-restricted epitope of *Listeria monocytogenes*. *Nature* **353**, 852-855.

Para, M.F., Baucke, R.B. & Spear, P.G. (1980). Immunoglobulin G(Fc)-binding receptors on virions of herpes simplex virus type 1 and transfer of these receptors to the cell surface by infection. *Journal of Virology* **34**, 512-520.

Parish, C.R. & McKenzie, I.F.C. (1978). A sensitive rosetting method for detecting subpopulations of lymphocytes which react with alloantisera. *Journal of Immunological Methods* **20**, 173-183.

Parnes, J., Velan, B., Felsenfeld, A., Ramanthan, L., Ferrini, U., Appella, E. & Seidman, J.G. (1981). Mouse β_2-microglobulin cDNA clones: a screening procedure for cDNA clones corresponding to rare mRNAs. *Proceedings of the National Academy of Sciences, USA* **78**, 2253-2257.

Parnes, J. & Seidman, J. (1982). Structure of wildtype and mutant mouse β-$_2$ microglobulin genes. *Cell* **29**, 661-669.

Parr, E.L. & McKenzie, I.F.C. (1979). Demonstration of Ia antigens on mouse intestinal epithelial cells by immunoferritin labelling. *Immunogenetics* **8**, 499-508.

Pass, R.F., Whitley, R.J., Whelchel, J.D., Diethelm, A.G., Reynolds, D.W. & Alford, C.A. (1979). Identification of patients with increased risk of infection with herpes simplex virus after renal transplantation. *Journal of Infectious Diseases* **140**, 487-492.

Pavan-Langston, D., Rong, B. & Dunkel, E.C. (1989). Extraneuronal herpetic latency: Animal and human corneal studies. *Acto Ophthalmologica (Copenhagen), supplement* **192**, 135-141.

Pellett, P.E., McKnight, J.L.C., Jenkins, F.J. & Roizman, B. (1985). Nucleotide sequence and predicted amino acid sequence of a protein encoded in a small herpes simplex virus DNA fragment capable of *trans*-inducing α genes. *Proceedings of the National Academy of Sciences, USA* **82**, 5870-5874.

Penfold, M.E., Armati, P. & Cunningham, A.L. (1994). Axonal transport of herpes simplex virions to epidermal cells: evidence for specialized mode of virus transport and assembly. *Proceedings of the National Academy of Sciences, USA* **91**, 6529-6533.

Pereira, R.A., Tscharke, D.C. & Simmons, A. (1994). Upregulation of class I major histocompatibility complex gene expression in primary sensory neurons, satellite cells and Schwann cells of mice in response to acute but not latent herpes simplex virus infection *in vivo*. *Journal of Experimental Medicine* **180**, 841-850.

Perng, G.C., Dunkel, E.C., Geary, P.A., Slanina, S.M., Ghiasi, H., Kaiwar, R., Nesburn, A.B. & Wechsler, S.L. (1994). The latency-associated transcript gene of herpes simplex virus type 1 (HSV-1) is required for efficient *in vivo* spontaneous reactivation of HSV-1 from latency. *Journal of Virology* **68**, 8045-8055.

Perry, L.J. & McGeoch, D.J. (1988). The DNA sequences of the long repeat region and adjoining parts of the long unique region in the genome of herpes simplex virus type 1. *Journal of General Virology* **69**, 2831-2846.

Peterslund, N.A. (1991). Herpesvirus infection: An overview of the clinical manifestations. *Scandanavian Journal of Infection- Supplement* **78**, 15-20.

Pfizenmaier, K., Jung, H., Starzinski-Powitz, A., Rollingnoff, M. & Wagner, H. (1977 a). The role of T-cells in anti-herpes simplex virus immunity. I. Induction of antigen specific cytotoxic T-lymphocytes. *Journal of Immunology* **119**, 939-944.

Pfizenmaier, K., Starzinski-Powitz, A., Rollingnoff, M., Falke, D. & Wagner, H. (1977 b). T-cell mediated cytotoxicity against herpes simplex virus-infected target cells. *Nature* **265**, 630-632.

Pinto, A.J., Stewart, D., van Rooijen, N. & Morahan, P.S. (1991). Selective depletion of liver and splenic macrophages using liposomes encapsulating the drug dichloromethylene diphosphate: effects on antimicrobial resistance. *Journal of Leukocyte Biology* **49**, 579-586.

Pircher, H., Groscurth, P., Baumhutter, S., Aguet M., Zinkernagel, R.M. & Hengartner, H. (1986). A monoclonal antibody against altered LFA-1 induces proliferation and lymphokine release of cloned T cells. *European Journal of Immunology* **16**: 172-181.

Ploegh, H.L., Cannan, E.L. & Strominger, J.L. (1979). Cell free translation of the mRNAs for heavy and light chains of HLA-A and HLA-B antigens. *Proceedings of the National Academy of Sciences, USA* **76**, 2273-2277.

Plummer, G. (1973). Isolation of herpesviruses from trigeminal ganglia of man, monkeys and cats. *Journal of Infectious Diseases* **128**, 345-347.

Poffenberger, K.L. & Roizman, B. (1985). Studies on non-inverting genome of a viable herpes simplex virus 1. Presence of head-to-tail linkages in packaged genomes and requirements for circularization after infection. *Journal of Virology* **53**, 589-595.

Posavad, C.M. & Rosenthal, K.L. (1992). Herpes simplex virus-infected human fibroblasts are resistant to and inhibit cytotoxic T-lymphocyte activity. *Journal of Virology* **66**, 6264-6272.

Post, L.E., Mackem, S. & Roizman, B. (1981). Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. *Cell* **24**, 555-565.

Potter, H., Weir, L. & Leder, P. (1984). Enhancer-dependent expression of human κ immunoglobulin genes introduced into mouse pre-beta lymphocytes by electroporation. *Proceedings of the National Academy of Sciences, USA* **81**, 7161-7165.

Potter, T.A., Rajan, T.V., Dick, R.F. & Bluestone, J.A. (1989). Substitution at residue 227 of H-2 class I molecules abrogates recognition by CD8-dependent, but not CD8-independent, cytotoxic T lymphocytes. *Nature* **337**, 73-75.

Powis, S., Townsend, A., Deverson, E., Bastin, J., Butcher, G. & Howard, J. (1991). Restoration of antigen presentation to the mutant cell line RMA-S by an MHC-linked transporter. *Nature* **354**, 528-531.

Powis, S., Mockridge, I., Kelly, A., Kerr, L., Glynne, R., Gileadi, U., Beck, S. & Trowsdale, J. (1992 a). Polymorphism in a second ABC transporter gene located within the class II region of the human MHC. *Proceedings of the National Academy of Sciences, USA* **89**, 1463-1467.

Preston, C.M. (1979 a). Abnormal properties of an immediate early polypeptide in cells infected with the herpes simplex virus type 1 mutant *ts*K. *Journal of Virology* **32**, 357-369.

Preston, C.M. (1979 b). Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant *ts*K. *Journal of Virology* **29**, 275-284.

Preston, C.M., Frame, M.C. & Campbell, M.E.M. (1988). A complex formed between cell components and an HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence. *Cell* **52**, 425-434.

Price, R.W., Katz, B.J. & Notkins, A.L. (1975). Latent infection of the peripheral ANS with herpes simplex virus. *Nature (London)* **257**, 686-688.

Price, J., Turner, D. & Cepko, C. (1987). Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. *Proceedings of the National Academy of Sciences, USA* **34**, 156-160.

Priola, S.A., Gustafson, D.P., Wagner, E.K. & Stevens, J.G. (1990). A major portion of the latent pseudorabies virus genome is transcribed in trigeminal ganglia of pigs. *Journal of Virology* **64**, 4755-4760.

Puga, A., Rosenthal, J.D., Openshaw, H. & Notkins, A.L. (1978). Herpes simplex virus DNA and mRNA sequences in acutely and chronically infected trigeminal ganglia of mice. *Virology* **89**, 102-111.

Quinn, T.C., Corey, L. & Chaffee, R.G. (1981). The etiology of anorectal infections in homosexual men. *Acyclovir Symposium, The American Journal of Medicine* **71**, 395-406.

Rabin, E.R., Jenson, A.B. & Melnick, J.L. (1968). Herpes simplex virus in mice: electron microscopy of neural spread. *Science* **162**, 126-127.

Rader, K.A., Ackland-Berglund, C.E., Miller, J.K., Pepose, J.S. & Leib, D.A. (1993). In vivo characterization of site-directed mutations in the promoter of the herpes simplex virus type 1 latency associated transcripts. *Journal of General Virology* **74**, 1859-1869.

Rager-Zisman, B. & Allison, A. C. (1976). Mechanism of immunological resistance to herpes simplex virus 1 (HSV-1) infection. *Journal of Immunology* **116**, 35-40.

Rajagopalan, S. & Brenner, M.B. (1994). Calnexin, retains unassembled major histocompatibility complex class I free heavy chains in the endoplasmic reticulum. *Journal of Experimental Medicine* **180**, 407-412.

Rajagopalan, S., Xu, Y. & Brenner, M.B. (1994). Retention of unassembled components of integral membrane proteins by calnexin. *Science* **263**, 387-390.

Rall, G.F., Mucke, L., Nerenberg, M. & Oldstone, M.B.A. (1994). A transgenic mouse model to assess the interaction of cytotoxic T lymphocytes with virally infected class I MHC-expressing astrocytes. *Journal of Neuroimmunology* **52**, 61-68.

Rall, G.F., Mucke, L. & Oldstone, M.B.A. (1995). Consequences of cytotoxic T lymphocyte interaction with major histocompatibility complex class I-expressing neurons *in vivo*. *Journal of Experimental Medicine* **182**, 1201-1212.

Ramshaw, I., Ruby, J., Ramsey, A., Ada, G. & Karupiah, G. (1992). Expression of cytokines by recombinant vaccinia viruses: a model for studying cytokines in virus infections *in vivo*. *Immunology Reviews* **127**, 157-182.

Raniero, D.E., Stasio, P. & Taylor, M.W. (1990). Specific effect of interferon on herpes simplex virus type 1 transactivation event. *Journal of Virology* **64**, 2588-2593.

Rattray, M.C., Corey, L., Reeves, W.C., Vontver, L.A. & Holmes, K.K. (1978). Recurrent genital herpes among women: symptomatic versus asymptomatic viral shedding. *British Journal of Venereal Disease* **54**, 262-265.

Rawls, W.E., Adam, E. & Melnick, J.L. (1972). Geographical variation in the association of antibodies to herpesvirus type 2 and carcinoma of the cervix. In: *Oncogenesis and Herpesviruses,* pp 424. Edited by Biggs P.M., de The G. & Paynes L.N. Scientific Publication II. Lyon, France: International Agency for Research on Cancer.

Rawls, W.E. (1985). Herpes simplex virus. In: *Virology,* pp527. Edited by Fields, B.N. 1st ed. New York: Raven Press.

Read, G.S. & Frenkel, N. (1983). Herpes simplex virus mutants in the virion associated shut-off of host polypeptide synthesis and exhibiting abnormal synthesis of α (immediate early) viral polypeptides. *Journal of Virology* **46**, 498-512.

Readhead, C., Popko, B., Takahashi, N., Shine, H.D., Saavedra, R.A., Sidman, R.L. & Hood, L. (1987). Expression of a myelin basic protein gene in transgenic shiverer mice: Correction of the dysmyelinating phenotype. *Cell* **48**, 703-712.

Rector, J.T., Lausch, R.N. & Oakes, J.E. (1982). Use of monoclonal antibodies for analysis of antibody-dependent immunity to ocular herpes simplex virus type 1 infection. *Infection and Immunity* **38**, 168-174.

Reddehase, M.J., Rothbard, J.B. & Koszinowski, V.H. (1989). A pentapeptide as minimal antigenic determinant for MHC class I-restricted T lymphocytes. *Nature* **337**, 651-653.

Reynolds, C.W. & Ortaldo, J.R. (1990). Nature and physiology of natural effector cells. In: *Immunophysiology: The role of cells and cytokines in immmunity and inflammation*, pp. 336-385. Edited by Oppenheim, J.J. & Shevach, E.M. New York: Oxford University Press.

Riordan, J.R., Rommens, J.M., Kerem, B.-T., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M.L., Iannuzzi, M.C., Collins, F.S. & Tsui, L.-C. (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245**, 1066-1073.

Roberts, M.S., Boundy, A., O'Hare, P., Pizzomo, M.C., Ciufo, D.M. & Hayward, G.S. (1988). Direct correlation between a negative autoregulatory response element at the cap site of the herpes simplex virus type 1 IE175 (α4) promoter and a specific binding site for the IE175 (ICP4) protein. *Journal of Virology* **62**, 4307-4320.

Rock, D.L. & Fraser, N.W. (1983). Detection of HSV-1 genome in central nervous system of latently infected mice. *Nature (London)* **302**, 523-525.

Rock, D.L. & Fraser, N.W. (1985). Latent herpes simplex virus type 1 DNA contains two copies of the virion DNA joint region. *Journal of Virology* **55**, 849-852.

Rock, D.L., Beam, S.L. & Mayfield, J.E. (1987 a). Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. *Journal of Virology* **61**, 3827-3831.

Rock, D.L., Nesburn, A.B., Ghiasi, H., Ong, J., Lewis, T.L., Lokensgard, J.R. & Wechsler, S.L. (1987 b). Detection of latency-related RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. *Journal of Virology* **61**, 3820-3826.

Rock, K.L., Gramm, C., Rothstein, L., Clark, K., Stein, R., Dick, L., Hwang, D. & Goldberg, A.L. (1994). Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* **78**, 761-771.

Roitt, I. (1988). Essential Immunology, 8th Edition. Oxford: Blackwell Scientific Publications.

Roizman, B. & Roane, P.R., Jnr. (1964). Multiplication of herpes simplex virus II. The relation between protein synthesis and the duplication of viral DNA in infected Hep-2 cells. *Virology* **22**, 262-269.

Roizman, B., Borman, G.S. & Kamali-Rousta, M. (1965). Macromolecular synthesis in cells infected with herpes simplex virus. *Nature (London)* **206**, 1374-1375.

Roizman, B. (1966). An inquiry into the mechanisms of recurrent herpes infections in man. In: *Perspectives in Virology*, pp. 283-304, Vol. 4 Edited by Pollard, M. New York: Harper & Row.

Roizman, B. & Furlong, D. (1974). The replication of herpesviruses. In: *Comprehensive Virology*, pp 229-403, Vol. 3 Edited by Fraenkel-conrat, H., Wagner, R.R. New York: Plenum Press.

Roizman, B. (1979 a). The structure and isomerization of herpes simplex virus genomes. *Cell* **16**, 481-494.

Roizman, B. (1979 b). The organization of the herpes simplex virus genomes. *Annual Reviews in Genetics* **13**, 25-57.

Roizman, B., Carmichael, L.E., Deinhardt, F., De The, G., Nahmias, A.J., Plowright, W., Rapp, F., Sheldrick, P., Takahashi, M. & Wolf, K. (1981). Herpesviridae: definition, provisional nomenclature, and taxonomy. *Intervirology* **16**, 201-217.

Roizman, B. (1990). Herpesviridae: A Brief Introduction. In: *Virology*, pp. 1787-1793, 2nd Ed. Edited by Fields, B.N., Knipe, D.M., *et al.* New York: Raven Press.

Roizman, B. & Sears, A.E. (1990). Herpes Simplex Viruses and their Replication. In: *Virology*, pp. 1795-1841, 2nd Ed. Edited by Fields, B.N., Knipe, D.M., *et al.* New York: Raven Press.

Romero, P., Corradin, G., Luescher, I.F. & Maryanski, J.L. (1991). H-2K^{d}-restricted antigenic peptides share a simple binding motif. *Journal of Experimental Medicine* **174**, 603-612.

Rose, J.K. & Doms, R.W. (1988). Regulation of protein export from the endoplasmic reticulum. *Annual Reviews of Cellular Biology* **4**, 257-288.

Rossol-Voth, R., Rossol, S., Schutt, K.H., Corridori, S., de Cian, W. & Falke, D. (1991). *In vivo* protective effect of tumour necrosis factor α against experimental infection with herpes simplex virus type 1. *Journal of General Virology* **72**, 143-147.

Rotem-Yehudar, R., Winograd, S., Sela, S., Coligan, J.E. & Ehrlich, R. (1994). Downregulation of peptide transporter genes in cell lines transformed with highly oncogenic adenovirus 12. *Journal of Experimental Medicine* **180**, 477-488.

Rothlein, R., Dustin, M.L., Mavlin, S.D. & Springer, T.A. (1986). A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *Journal of Immunology* **137**, 1270-1274.

Russell, W.C. (1962). A sensitive and precise assay for herpes virus. *Nature (London)* **195**, 1028-1029.

Sachs, D.H. & Cone, J.L. (1973). A mouse B cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *Journal of Experimental Medicine* **138**, 1289-1304.

Sacks, W.R., Greene, C.C., Aschman, D.P. & Schaffer, P.A. (1985). Herpes simplex virus type 1 ICP27 is an essential regulatory protein. *Journal of Virology* **55**, 796-805.

Sacks, W.R. & Schaffer, P.A. (1987). Deletion mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein ICP0 exhibit impaired growth in cell culture. *Journal of Virology* **61**, 829-839.

Sainz de la Maza, W., Wells, P.A. & Foster, C.S. (1989). Cyclic nucleotide modulation of herpes simplex virus latency and reactivation. *Investigative Opthalmology Visual Science* **30**, 2154-2159.

Salahuddin, S.Z., Ablashi, D.V., Markham, P.D., Josephs, S.F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Staal, F., Kramarsky, B. & Gallo, R.C. (1986). Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* **234**, 596-601.

Salter, R.D., Howell, D.N. & Cresswell, P. (1985). Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. *Immunogenetics* **21**, 235-246.

Salter, R.D. & Cresswell, P. (1986). Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. *European Molecular Biological Organization* **5**, 943-949.

Salter, R.D., Norment, A.M., Chen, B.P., Clayberger, C., Krensky, A.M., Littman, D.R. & Parham, P. (1989). Polymorphism in the alpha 3 domain of HLA-A molecules affects binding to CD8. *Nature* **338**, 345-347.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Sandrin, M.S., Potter, T.A., Morgan, G.M. & McKenzie, I.F.C. (1978). Detection of mouse alloantibodies by rosetting with protein A-coated sheep red blood cells. *Transplantation* **26**, 126-130.

Sanes, J.R., Rubenstein, J.L.R. & Nicholas, J.F. (1983). Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. *European Molecular Biological Organization* **5**, 3133-3142.

Saper, M., Bjorkman, P. & Wiley, D. (1991). Refined structure of the human histocompatibility antigen HLA-A2 and 2.6A resolution. *Journal of Molecular Biology* **219**, 277-319.

Sarmiento, M., Haffey, M. & Spear, P.G. (1979). Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7 (B2) in virion infectivity. *Journal of Virology* **29**, 1149-1158.

Sawtell, N.M. & Thompson, R.L. (1992). Herpes simplex virus type 1 latency-associated transcription unit promotes anatomical site-dependent establishment and reactivation from latency. *Journal of Virology* **66**, 2157-2166.

Schachner, M.G. & Siedman, R.L. (1973). Distribution of H-2 alloantigen in adult and developing mouse brains. *Brain Research* **60**, 191-198.

Schachner, M.G. & Hammerling, U. (1974). The postnatal development of antigens on mouse brain cell surfaces. *Brain Research* **73**, 362-371.

Schalling, M., Ekman, M., Kaaya, E.E., Linde, A. & Biberfeld, P. (1995). A role for a new herpesvirus (KSHV) in different forms of Kaposi's sarcoma. *Nature Medicine* **1**, 707-708.

Schimeld, C., Tullo, A.B., Easty, D.L. & Thomsitt, J. (1982). Isolation of HSV from the cornea in chronic stromal keratitis. *British Journal of Ophthlamology* **66**, 643-647.

Schmid, D.S. & Rouse, B.T. (1992). The role of T cell immunity in control of herpes simplex virus. In: *Herpes Simplex Viruses: Pathogenesis, Immunobiology and Control*, pp 57-74. Edited by Rouse, B.T. Berlin: Springer-Verlag.

Schmidt, O.W., Fife, K.H. & Corey, L. (1984). Reinfection is an uncommon occurence in patients with symptomatic recurrent genital herpes. *Journal of Infectious Diseases* **149**, 645-646.

Schneweis, K.E. & Brandis, H. (1961). Typendifferenzen beim herpes simplex virus. *Zentralbl Bakteriol Paraitenkunde Infektionskr* **183**, 556-558.

Schulz, M., Aichele, P., Schneider, R., Hansen, T.H., Zinkernagel, R.M. & Hengarther, H. (1991). Major histocompatibility complex binding and T cell recognition of a viral nonapeptide containing a minimal tetrapeptide. *European Journal of Immunology* **21**, 1181-1185.

Schumacher, T.N., DeBruijn, M.L.H., Vernie, L.N., Kast, W.M., Melief, C.J.M., Neefjes, J.J. & Ploegh, H.L. (1991). Peptide selction by MHC class I molecules. *Nature* **350**, 703-706.

Schumacher, T.N.M., Kantesaria, D.V., Heemels, M.T., Ashton-Rickardt, P.G., Sheperd, J.C., Fruh, K., Yang, Y., Peterson, P.A., Tonegawa, S. & Ploegh, H.L. (1994). Peptide length and sequence specificity of the mouse tap1/tap2 translocator. *Journal of Experimental Medicine* **179**, 533-540.

Schwartz, B.D., Paul, W.E. & Shevach, E.M. (1976). Guinea-pig Ia antigens; functional significance and chemical characterization. *Transplantation Reviews* **30**, 174-196.

Scriba, M. (1977). Extraneural localisation of herpes simplex virus in latently infected guinea pigs. *Nature (London)* **267**, 529-531.

Scriba, M. (1981). Persistence of herpes simplex virus (HSV) infection in ganglia and peripheral tissues of guinea pigs. *Medical Microbiology and Immunology (Berlin)* **169**, 91-96.

Sears, A.E., Halliburton, I.W., Meignier, B., Silver, S. & Roizman, B. (1985 a). Herpes simplex virus 1 mutant deleted in the α22 gene: growth and gene expression in permissive and restrictive cells and establishment of latency in mice. *Journal of Virology* **55**, 338-346.

Sears, A.E., Meignier, B. & Roizman, B. (1985 b). Establishment of latency in mice by herpes simplex virus 1 recombinants that carry insertions affecting regulation of the thymidine kinase gene. *Journal of Virology* **55**, 410-416.

Sedarati, F., Izumi, K.M., Wagner, E.K. & Stevens, J.G. (1989). Herpes simplex virus type 1 latency-associated transcription plays no role in establishment or maintenance of a latent infection in murine sensory neurons. *Journal of Virology* **63**, 4455-4458.

Sedarati, F., Margolis, T.P. & Stevens, J.G. (1993). Latent infection can be established with drastically restricted transcription and replication of the HSV-1 genome. *Virology* **192**, 687-691.

Sekulovich, R., Leary, K. & Sandri-Goldin, R.M. (1988). The herpes simplex virus type 1 α protein ICP27 can act as a *trans*-repressor or a *trans*-activator in combination with ICP0 and ICP4. *Journal of Virology* **62**, 4510-4522.

Selling, B. & Kibrick, S. (1964). An outbreak of herpes simplex among wrestlers (herpes gladiatorium). *New England Journal of Medicine* **270**, 979-982.

Serge, K., Rask, L. & Peterson, P.A. (1981). Role of β_2-microglobulin in the intracellular processing of HLA antigens. *Biochemistry* **20**, 4523-4530.

Sethi, K., Ometa, K. & Schneweis, K.E. (1983). Protection of mice from fatal herpes simplex virus type 1 infection by adoptive transfer of cloned virus-specific and H-2 restricted cytotoxic T lymphocytes. *Journal of General Virology* **64**, 443-447.

Severin, M.J. & White, R.J. (1968). The neural transmission of herpes simplex virus in mice. Light and electron microscopic findings, *American Journal of Pathology* **53**, 1009-1020.

Severini, A., Morgan, R.A., Tovell, D.R. & Tyrrell, D.L.J. (1994). Study of the structure of replicative intermediates of HSV-1 DNA by pulse-field gel electrophoresis. *Virology* **200**, 428-435.

Severinsson, L. & Peterson, P.A. (1984). β_2-microglobulin induces intracellular transport of human class I transplantation antigen heavy chains in *Xenopus laevis* oocytes. *Journal of Cell Biology* **99**, 226-232.

Shaw, S., Luce, G.E., Quinones, R., Gress, R.E., Springer, T.A. & Sanders, M.E. (1986). Two antigen-independent adhesion pathways used by cytotoxic T-cell clones. *Nature* **323**, 262-264.

Shaw, S. & Luce, G.E. (1987). The lymphocyte function associated antigen (LFA)-1 and CD2/LFA-3 pathways of antigen independent human T cell adhesion. *Journal of Immunolgy* **139**, 1037-1045.

Shawar, S.M., Vyas, J.M., Rodgers, J.R. & Rich, R.R. (1994). Antigen presentation by major histocompatibility complex class I-B molecules. *Annual Review of Immunology* **12**, 839-880.

Sheldrick, P. & Berthelot, N. (1975). Inverted repetitions in the chromosome of herpes simplex virus. *Cold Spring Harbor Symposium on Quantitative Biology* **39**, 667-678.

Shepherd, J.C., Schumacher, T.N.M., Ashton-Rickardt, P.G., Imaeda, S., Ploegh, H.L., Janeway, C.A. & Tonegawa, S. (1993). Tap1-dependent peptide translocation *in vitro* is ATP-dependent and peptide selective. *Cell* **74**, 577-584.

Sherry, M.K., Klainer, A.S., Wolff, M. & Gerhard, H. (1988). Herpetic tracheobronchitis. *Annals of Internal Medicine* **109**, 229-233.

Shieh, M.-T., WuDunn, D., Montgomery, R.I., Esko, J.D. & Spear, P.G. (1992). Cell surface receptors for herpes simplex virus are heparan sulphate proteoglycans. *The Journal of Cell Biology* **116**, 1273-1281.

Ship, I.I., Brightman, V.J. & Laster, L.L. (1967). The patient with recurrent aphthous ulcers and the patient with recurrent herpes labialis: a study of two population samples. *Journal of the American Dental Association* **75**, 645-654.

Shreffler, D.C. & David, C.S. (1975). The H-2 complex major histocompatibility complex and the Ia immune response region: Genetic variation, function and organization. *Advances in Immunology* **20**, 125-195.

Silver, S. & Roizman, B. (1985). *gamma*$_2$ Thymidine kinase chimeras are identically transcribed but regulated as *gamma*$_2$ genes in herpes simplex virus genomes and as *beta* genes in cell genomes. *Molecular and Cellular Biology* **5**, 518-528.

Simmons, A. & Nash, A.A. (1984). Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. *Journal of Virology* **52**, 816-821.

Simmons, A. (1985) *Pathogenesis and immunology of herpes simplex virus infection of mice.* (Doctor of Philosophy dissertation), University of Cambridge, UK.

Simmons, A. & Nash, A.A. (1985). Role of antibody in primary and recurrent herpes simplex virus infection. *Journal of Virology* **53**, 944-948.

Simmons, A. & Nash, A.A. (1987). Effect of B cell suppression on primary infection and reinfection of mice with herpes simplex infection. *Journal of Infectious Diseases* **155**, 649-654.

Simmons, A. (1989). H-2 linked genes influence the severity of herpes simplex virus infection of the peripheral nervous system. *Journal of Experimental Medicine* **169**, 1503-1507.

Simmons, A. & La Vista, A.B. (1989). Neural infection in mice after cutaneous inoculation with HSV-1 is under complex host genetic control. *Virus Research* **13**, 263-270.

Simmons, A. & Tscharke, D.C. (1992). Anti-CD8 impairs clearance of herpes simplex virus from the nervous system: Implications for the fate of virally infected neurons. *Journal of Experimental Medicine* **175**, 1337-1344.

Simmons, A., Slobedman, B., Speck, P., Arthur, J. & Efstathiou, S. (1992 a). Two patterns of persistence of herpes simplex virus DNA sequences in the nervous system of latently infected mice. *Journal of General Virology* **73**, 1287-1291.

Simmons, A., Tscharke, D. & Speck, P. (1992 b). The role of immune mechanisms in control of Herpes simplex virus infection of the peripheral nervous system. In: *Herpes simplex virus: Pathogenesis, immunobiology and control,* pp. 31-56. Edited by Rouse, B.T. Berliin: Springer-Verlag.

Skias, D.D., Kim, D.K., Reder, A.T., Antel, J.P., Lancki, D.W. & Fitch, F.W. (1987). Susceptibility of astrocytes to class I MHC antigen-specific cytotoxicity. *Journal of Immunology* **138**, 3254-3258.

Slobedman, B., Efstathiou, S. & Simmons, A. (1994). Quantitative analysis of herpes simplex virus DNA and transcriptional activity in ganglia of mice latently infected with wildtype and thymidine kinase deficient virus strains. *Journal of General Virology* **75**, 2469-2474.

Smiley, J.R., Fong, B. & Leung, W.-C. (1981). Construction of a double-jointed herpes simplex virus DNA molecule: inverted repeats are required for segment inversion and direct repeats promote deletions. *Virology* **113**, 345-362.

Smiley, J.R., Lavery, C. & Howes, M. (1992). The herpes simplex virus type 1 (HSV-1) *a* sequence serves as a cleavage/packaging signal but does not drive recombinational genome isomerization when it is inserted into the HSV-2 genome. *Journal of Virology* **66**, 7505-7510.

Smith, I.L., Hardwicke, M.A. & Sandri-Goldin, R.M. (1992). Evidence that the herpes simplex virus immediate early protein ICP27 acts post-transcriptionally during infection to regulate gene expression. *Virology* **186**, 74-86.

Smith, P.M., Wolcott, R.M., Chervenak, R. & Jennings, S.R. (1994). Control of acute cutaneous herpes simplex virus infections: T cell-mediated viral clearance is dependent upon interferon-γ (IFN-γ). *Virology* **202**, 76-88.

Smith, R.L., Escudero, J.L. & Wilcox, C.L. (1994 b). Regulation of the herpes simplex virus latency-associated transcript durin establishment of latency in sensory neurons *in vitro*. *Virology* **202**, 49-60.

Smith, R.L., Geller, A.I., Escudero, K.W. & Wilcox, C.L. (1995). Long-term expression in sensory neurons in tissue culture from herpes simplex virus type 1 (HSV-1) promoters in an HSV-1 derived vector. *Journal of Virology* **69**, 4593-4599.

Snell, G.D., Dausset, J. & Nathenson, S. (1975). Histocompatibility. New York: Academic Press.

Sodoyer, R., Damotte, M., Delovitch, T.L., Trucy, J., Jordan, B.R. & Strachan, T. (1984). Complete nucleotide sequence of a gene encoding a functional human class I histocompatibility antigen (HKA-Cw3). *European Molecular Biological Organization* **3**, 879-885.

Sompayrac, L.M. & Danna, K.J. (1981). Efficient infection of mammalian cells with SV40 DNA. *Proceedings in the National Academy of Sciences, USA* **78**, 7575-7578.

Southern, E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503-517.

Spaete, R. & Frenkel, N. (1982). The herpes simplex virus amplicon: a new eukaryotic defective-virus cloning-amplifying vector. *Cell* **30**, 295-304.

Spaete, R.R. & Frenkel, N. (1985). The herpes simplex virus amplicon: Analysis of *cis*-acting replication functions. *Proceedings of the National Academy of Sciences, USA* **82**, 694-698.

Spear, P.G. & Roizman, B. (1972). Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirus. *Journal of Virology* **9**, 143-159.

Spear, P.G. (1993). Entry of alphaherpesviruses into cells. *Seminars in Virology* **4**, 167-180.

Speck, P.G. & Simmons, A. (1991). Divergent molecular pathways of productive and latent infection with a virulent strain of herpes simplex virus type 1. *Journal of Virology* **65**, 4001-4005.

Speck, P.G. & Simmons, A. (1992). Synchronous appearance of antigen-positive and latently infected neurons in spinal ganglia of mice infected with a virulent strain of herpes simplex virus. *Journal of General Virology* **73**, 1281-1285.

Spector, D., Purves, F. & Roizman, B. (1991). Role of α-transinducing factor (VP16) in the induction of α genes within the context of viral genomes. *Journal of Virology* **65**, 3504-3513.

Spies, T., Bresnahan, M., Bahram, S., Arnold, D., Blanck, E., Mellins, E., Pious, D. & DeMars, R. (1990). A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. *Nature* **348**, 744-747.

Spies, T. & DeMars, R. (1991). Restored expression of major histocompatibility class I molecules by gene transfer of a putative peptide transporter. *Nature* **351**, 323-324.

Spies, T., Cerundolo, V., Colonna, M., Cresswell, P., Townsend, A. & DeMars, R. (1992). Presentation of viral antigen by MHC class I molecules is dependent on a putative peptide transporter heterodimer. *Nature* **355**, 644-646.

Spits, H., Van Schooten W., Keizer, H., Van Seventer, G., Van De Rijn, M., Terhorst, C. & de Vries, J.E. (1986). Alloantigen recognition is preceded by nonspecific adhesion of cytotoxic T cells and target cells. *Science* **232**, 403-405.

Spivack, J.G. & Fraser, N.W. (1987). Detection of herpes simplex virus type 1 transcripts during latent infection in mice. *Journal of Virology* **61**, 3841-3847.

Spivack, J.G. & Fraser, N.W. (1988 a). Expression of herpes simplex virus type 1 (HSV-1) latency-associated transcripts and transcripts affected by the deletion in avirulent mutant HFEM: evidence for a new class of HSV-1 genes. *Journal of Virology* **62**, 3281-3287.

Spivack, J.G. & Fraser, N.W. (1988 b). Expression of herpes simplex virus type 1 latency-associated transcripts in the trigeminal ganglia of mice during acute infection and reactivation of latent infection. *Journal of Virology* **62**, 1479-1485.

Spivack, J.G., Woods, G.M. & Fraser, N.W. (1991). Identification of a novel latency-specific splice donor signal within the herpes simplex virus type 1 2.0 kilobase latency-associated transcript (LAT): translation inhibition of LAT open reading frames by the intron within the 2.0 kilobase LAT. *Journal of Virology* **65**, 6800-6810.

Sprent, J. (1978). Restricted helper function of F1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for the restrictions affecting helper cells induction and T-B collaboration, both mapping to the K end of the H-2 complex. *Journal of Experimental Medicine* **147**, 1159-1174.

Springer, T.A. (1990). The sensation and regulation of interactions with the extracellular enviroment: the cell biology of lymphocyte adhesion receptors. *Annual Reviews in Cell Biology* **6**, 359-403.

Stagno, H. & Whitley, R.J. (1985). Herpesvirus infections of pregnancy. *New England Journal of Medicine* **313**, 1327-1330.

Stannard, L.M., Fuller, A.O. & Spear, P.G. (1987). Herpes simplex virus glycoproteins associated with different morphological entities projecting from the virion envelope. *Journal of General Virology* **68**, 715-725.

Steiner, I., Spivack, J.G., O'Boyle, D.R.I.I., Lavi, E. & Fraser, N.W. (1988). Latent herpes simplex virus type 1 transcription in human trigeminal ganglia. *Journal of Virology* **62**, 3493-3496.

Steiner, I., Spivack, J.G., Lirette, R.P., Brown, S.M., MacLean, A.R., Subak-Sharpe, J.H. & Fraser, N.W. (1989). Herpes simplex virus type 1 latency-associated transcripts are evidently not essential for latent infection. *European Molecular Biological Organization* **8**, 505-511.

Steiner, I., Spivack, J.G., Deshmane, S.L., Ace, C.I., Preston, C.M. & Fraser, N.W. (1990). A herpes simplex virus type 1 mutant containing a nontransinducing Vmw65 protein establishes latent infection *in vivo* in the absence of viral replication and reactivates efficiently from explanted trigeminal ganglia. *Journal of Virology* **64**, 1630-1638.

Steinman, R.M. (1981). Dendritic cells. *Transplantation* **31**, 151-155.

Steinmetz, M., Moore, K.W., Frelinger, J.G., Sher, B.T., Shen, F.W., Boyse, E.A. & Hood, L. (1981). A pseudogene homologous to mouse transplantation antigens: transplantation antigens are encoded by eight exons that correlate with protein domains. *Cell* **25**, 683-692.

Steinmetz, M., Winoto, A., Minard, K. & Hood, L. (1982). Clusters of genes encoding mouse transplantation antigens. *Cell* **28**, 489-498.

Stephanopoulos, D.E., Kappes, J.C. & Bernstein, D.I. (1988). Enhanced *in vitro* reactivation of herpes simplex virus type 2 from latently infected guinea pig neural tissues by 5-azacytidine. *Journal of General Virology* **69**, 1079-1083.

Stern, S., Tananka, M. & Herr, W. (1989). The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. *Nature (London)* **341**, 624-630.

Sternberger, L.A. (1979). *Immunochemistry*: Second Edition. New York: John Wiley and Sons.

Stevens, J.G. & Cook, M.L. (1971). Latent herpes simplex virus in spinal ganglia of mice. *Science* **173**, 843-845.

Stevens, J.G., Nesburn, A.B. & Cook, M.L. (1972). Latent herpes simplex virus from trigeminal ganlia of rabbits with recurrent eye infection. *Nature (New Biol.)* **235**, 216-217.

Stevens, J.G., Wagner, E.K., Devi-Rao, G.B., Cook, M.L. & Feldman, L.T. (1987). RNA complementary to a herpesvirus α gene mRNA is prominent in latently infected neurons. *Science* **235**, 1056-1059.

Stevens, J.G. (1989). Human herpesviruses: a consideration of the latent state. *Microbiological Reviews* **53**, 318-332.

Stow, N.D. (1982). Localization of an origin of DNA replication within the TRs/IRs repeated region of the herpes simplex virus type 1 genome. *European Molecular Biological Organization* **1**, 863-867.

Stow, N.D. & McMonagle, E.D. (1982). Propagation of foreign DNA sequences linked to a herpes simplex virus origin of replication. In *Eukaryotic Viral Vectors.* Edited by Gluzman, Y. New York: Cold Spring Harbor Laboratory Press.

Stow, N.D. & McMonagle, E.C. (1983). Characterization of the TRs/IRs origin of DNA replication of herpes simplex virus type 1. *Virology* **130**, 427-438.

Stow, N.D., McMonagle, E.C. & Davison, J. (1983). Fragments from both termini of the Herpes simplex virus type 1 genome contain signals required for the encapsidation of viral DNA. *Virology* **11**, 8205-8220.

Stow, N.D. & Stow, E.C. (1986). Isolation and characterization of a herpes simplex virus type 1 mutant containing a deletion with the gene encoding the immediate early polypeptide Vmw110. *Journal of General Virology* **67**, 2571-2585.

Strachan, T., Sodoyer, R., Damotte, M. & Jordan, B.R. (1984). Complete nucleotide sequence of a functional class I HLA gene, HLA-A3: implications for the evolution of HLA genes. *European Molecular Biological Organization* **3**, 887-894.

Stroop, W.G., Rock, D.L. & Fraser, N.W. (1984). Localization of herpes simplex virus in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infections by *in situ* hybridization. *Laboratory Investigation* **51**, 27-38.

Su I.J., Hsu, Y.S., Chang, Y.C. & Wang, I.W. (1995). Herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma from AIDS and non-AIDS patients in Taiwan. *Lancet* **345**, 722-723.

Subak-Sharpe, J.H., Al-Saadi, S.A. & Clements, G.B. (1984). Herpes simplex virus type 2 establishes latency in the mouse footpad and sensory ganglia. *Journal of Investigative Dermatology, supplement 1* **83**, 67-71.

Suh, W.-K., Cohen-Doyle, M.F., Fruh, K., Wang, K., Peterson, P.A. & Williams, D.B. (1994). Interaction of MHC class I molecules with the transporter associated with antigen processing. *Science* **264**, 1322-1326.

Sutherland, J., Mannoni, P., Rosa, F., Huyat, D., Turner, R.A. & Fellous, M. (1985). Induction of the expression of HLA class I antigens on K562 cells by interferons and sodium butyrate. *Human Immunology* **12**, 65-73.

Suzak, S. & Martin, J.R. (1989). Herpes simplex virus type 2 transcripts in trigeminal ganglia during acute and latent infection in mice. *Journal of Neurological Science* **93**, 239-251.

Suzumura, A. & Silberberg, D.H. (1985). Expression of H-2 antigen on oligodendrocytes is induced by soluble factors from concanavalin activated T cells. *Brain Research* **336**, 171-175.

Suzumura, A., Lavi, E., Weiss, S.R. & Silberberg, D.H. (1986). Cornavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. *Science* **232**, 991-993.

Swain, S.L. (1983). T cell subsets and the recognition of MHC class. *Immunology Reviews* **74**, 129-142.

Swanson, L.W., Simmons, D.M., Arriza, J., Hammer, R., Brinster, R., Rosenfeld, M.G. & Evans, R.M. (1985). Novel developmental specificity in the nervous system of transgenic animals expressing growth hormone fusion genes. *Nature* **317**, 363-366.

Tada, N., Kimura, S., Hatzfeld, A. & Hammerling, U. (1980). Ly-m11: The H-3 region of mouse chromosome 2 controls a new cell surface alloantigen. *Immunogenetics* **11**, 441-449.

Takei, F. (1985). Inhibition of mixed lymphocyte response by a rat monoclonal antibody to a novel murine lymphocyte activation antigen. *Journal of Immunology* **134**, 1403-1407.

Tenser, R.B. & Dunstan, M.E. (1979). Herpes simplex virus thymidine kinase expression in infection of the trigeminal ganglion. *Virology* **99**, 417-422.

Tenser, R.B., Miller, R.L. & Rapp, F. (1979). Trigeminal ganglion infection by thymidine kinase negative mutants of herpes simplex virus. *Science* **205**, 915-917.

Tenser, R.B., Ressel, S. & Dunstan, M.E. (1981). Herpes simplex virus thymidine kinase expression in trigeminal ganglion infection: Correlation of enzyme activity with virus titer and evidence of *in vivo* complementation. *Virology* **112**, 328-341.

Tenser, R.B., Dawson, M., Ressel, S.J. & Dunstan, M.E. (1982). Detection of herpes simplex virus mRNA in latently infected trigeminal ganglion neurons by *in situ* hybridization. *Annals of Neurology* **11**, 285-291.

Tenser, R.B., Hay, K.A. & Edris, W.A. (1989). Latency associated transcript but not reactivatable virus is present in sensory ganglion neurons after inoculation of thymidine kinase-negative mutants of herpes simplex virus type 1. *Journal of Virology* **63**, 2861-2865.

Tenser, R.B., Edris, W.A., Hay, K.A. & de Galan, B.E. (1991). Expression of herpes simplex virus type 2 latency-associated transcript in neurons and non-neurons. *Journal of Virology* **65**, 2745-2750.

Tewarson, S., Zaleska Rutczynska, Z., Figueroa, F. & Klein, J. (1983). Polymorphism of Qa and Tla loci of the mouse. *Tissue antigens* **22**, 204-212.

Thale, R., Szepan, U., Hengel, H., Geginat, G., Lucin, P. & Koszinowski, U.H. (1995). Identification of the mouse cytomegalovirus genomic region affecting major histocompatibility complex class I molecule transport. *Journal of Virology* **69**, 6098-6105.

Tiefenthaler, G., Hünig, T., Dustin, M.L., Springer, T.A. & Meuer, S.C. (1987). Purified lymphocyte function-associated antigen-3 and T11 target structure are active in CD2-mediated T cell stimulation. *European Journal of Immunology* **17**, 1847-50.

Timbury, M.C. (1993). Herpesviridae: herpes simplex and varizella-zoster virus infections. In: *Principles of Bacteriology, Virology and Immunity*, pp. 430-441, 8th Ed., Vol. 4 Edited by Collier, L.H. & Tombury, M.C. London: Topley & Wilson.

Tine, J.A., Walsh, A., Rathbun, D., Leonard, L., Wakeland, E.K., Dilwith, R. & Flaherty, L. (1990). Genetic polymorphisms of Q region genes from wild-derived mice: implications for Q region evolution. *Immunogenetics* **31**, 315-325.

Tognon, M., Furlong, D., Conley, A.J. & Roizman, B. (1981). Molecular genetics of herpes simplex virus. V. Characterization of a mutant defective in ability to form plaques at low temperatures and in a viral function which prevents accumulation of coreless capsids at nuclear pores late in infection. *Journal of Virology* **40**, 870-880.

Torseth, J.W. & Merigan, C.T. (1986). Significance of local γ-interferon production in recurrent herpes simplex virus infection. *Journal of Infectious Diseases* **153**, 979-984.

Townes, T.M., Lingrel, J.B., Chen, H.Y., Brinster, R.L. & Palmiter, R.D. (1985). Erythroid-specific expression of human beta-globin genes in transgenic mice. *EMBO* **4**, 1715-1723.

Townsend, A.R.M., McMichael, A.J., Carter, N.P., Huddleston, J.A. & Brownlee, G.G. (1984). Cytotoxic T cell recognize fragments of the influenza nucleoprotein & hemagglutinin expressed in transfected mouse L cells. *Cell* **39**, 13-25.

Townsend, A.R.M., Gotch, F.M. & Davey. J. (1985). Cytotoxic T cells recognise fragments of the influenza nucleoprotein. *Cell* **42**, 457-467.

Townsend, A.R.M., Rothbard, J., Gotch, F.M., Bahadur, G., Wraith, D. & McMichael, A.J. (1986 a) The epitopes of influenza nucleoprotien recognized by cytotoxic T lymphocytes can be defined by short peptides. *Cell* **44**, 959-968.

Townsend, A., Bastin, J., Gould, K. & Brownlee, G. (1986 b). Cytotoxic T lymphocytes recognize influenza hemagglutinin that lacks a signal sequence. *Nature* **234**, 575-577.

Townsend, A., Bastin, J., Gould, K., Brownlee, G., Andrew, M., Coupar, D., Boyle, D., Chan, S. & Smith, G. (1988). Defective presentation to class I restricted cytotoxic T lymphocytes in vaccinia-infected cells is overcome by enhanced degradation of antigen. *Journal of Experimental Medicine* **168**, 1211-1218.

Townsend, A., Ohlen, C., Bastin, J., Ljunggren, H-G., Foster, L. & Karre, K. (1989). Association of class I major histocompatibility heavy and light chains induced by viral peptides. *Nature* **340**, 443-448.

Townsend, A. & Trowsdale, J. (1993). The transporteres associated with antigen presentation. *Seminars in Cell Biology* **4**, 53-61.

Trousdale, M., Steiner, I., Spivack, J.G., Deshmane, S.L., Brown, S.M., MacLean, A.C., Subak-Sharpe, J. & Fraser, N.W. (1991). Evidence that the herpes simplex virus type 1 latency associated transcripts play a role in reactivation of latent infections in vivo. *Journal of Virology* **65**, 6989-6993.

Trowsdale, J., Hanson, I., Mockridge, I., Beck, S., Townsend, A. & Kelly, A. (1990). Sequences encoded in the clas II region of the MHC related to the "ABC" superfamily of transporters. *Nature* **348**, 741-744.

Tullo, A.B., Shimeld, C., Blyth, W.A., Hill, T.J. & Easty, D.L. (1982). Spread of virus and distribution of latent infection following ocular herpes simplex in the non-immune and immune mouse. *Journal of General Virology* **63**, 95-101.

Turner, D.L. & Cepoka, C. (1987). Cell lineage in the rat retina: A common progenitor for neurons and glia persists late in development. *Nature* **328**, 131-136.

Udaka, K., Tsomides, T.J., Walden, P., Fukusen, N. & Eisen, H.N. (1993). A ubiquitous protein is the source of naturally occurring peptides that are recognized by $CD8^+$ T-cell clone. *Proceedings of the National Academy of Sciences, USA* **90**, 11272-11276.

Urban, R.G., Chicz, R.M., Lane, W.S., Strominger, S., Rehm, J.L., Kenter, M.J.H., UytdeHaag, F.G.C.M., Ploegh, H., Uchanska-Ziegler, B. & Ziegler, A. (1994). A subset of HLA-B27 molecules contains peptides much longer than nanomers. *Proceedings of the National Academy of Sciences, USA* **91**, 1534-1538.

Valyi-Nagy, T., Deshmane, S.L., Spivack, J.G., Steiner, I., Ace, C.I., Preston, C.M. & Fraser, N.W. (1991). Investigation of herpes simplex virus type 1 (HSV-1) gene expression and DNA synthesis during the establishment of latent infection by an HSV-1 mutant, *in*1814, that does not replicate in mouse trigeminal ganglia. *Journal of General Virology* **72**, 641-649.

Van Noesel, C., Miedema, F., Brouwer, M., deRie, M.A., Aarden, L.A. & Van Lier, R.A.W. (1988). Regulatory properties of LFA-1 alpha and beta chains in human T-lymphocyte activation. *Nature* **333**, 850-55.

Van Bleek, G.M. & Nathenson, S.G. (1990). Isolation of endogenously processed immunodominant viral peptide from the class I $H\text{-}2K^b$ molecule. *Nature* **348**, 213-216.

Van Seventer, G.A., Shimizu, Y., Horgan, K.J. & Shaw, S. (1990). The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. *Journal of Immunology* **144**, 4579-4586.

Van Kaer, L., Ashton-Rickardt, P.G., Ploegh, H.L. & Tonegawa, S. (1992). Tap1 mutant mice are deficient in antigen presentation, surface class I molecules and $CD4^-CD8^+$ T cells. *Cell* **71**, 1205-1214.

Van Kaer, L., Ashton-Rickardt, P.G., Eichelberger, M., Gaczynska, M., Nagashima, K., Rock, K.L., Goldberg, A.L., Doherty, P.C. & Tonegawa, S. (1994). Altered peptidase and viral specific T cell response in LMP2 mutant mice. *Immunity* **1**, 533-541.

Varmuza, S.L. & Smiley, J.R. (1985). Signals for site-specific cleavage of herpes simplex virus DNA: maturation involves two separate cleavage events at sites distal to the recognition site. *Cell* **41**, 792-802.

Vitetta, E.S. & Capra, J.D. (1978). The protein products of the murine 17th chromosome: genetics and structure. *Advances in Immunology* **26**, 147-193.

Vlazny, D.A. & Frenkel, N. (1981). Replication of herpes simplex virus DNA: location of replication recognition signals within the defective virus genomes. *Proceedings of the National Academy of Sciences, USA* **78**, 742-746.

Vlazny, D.A., Kwong, A. & Frenkel, N. (1982). Site specific cleavage packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full length viral DNA. *Proceedings of the National Academy of Sciences, USA* **79**, 1423-1427.

Wada, I., Rindress, D., Cameron, P., Ou, W., Doherty, W.-J., Louvard, D., Bell, A., Dignard, D., Thomas, D. & Bergeron, J.J. (1991). SSR alpha and associated calnexin are major calcium binding proteins of the endoplasmic reticulum membrane. *Journal of Biological Chemistry* **266**, 19599-19610.

Wadsworth, S., Jacob, R.J. & Roizman, B. (1975). Anatomy of herpes simplex virus DNA. II. Size, composition and arrangement of inverted terminal repetitions. *Journal of Virology* **15**, 1487-1497.

Wagner, H.M. & Summers, W.C. (1978). Structure of the joint region and the termini of the DNA of herpes simplex virus type 1. *Journal of Virology* **27**, 374-387.

Wagner, E.K., Devi-Rao, G., Feldman, L.T., Dobson, A.T., Zhang, Y.-F., Flanagan, W.M. & Stevens, J.G. (1988 a). Physical characterization of the herpes simplex virus latency-associated transcript in neurons. *Journal of Virology* **62**, 1194-1202.

Wagner, E.K., Flanagan, W.M., Devi-Rao, G., Zhang, Y.-F., Hill, J.M., Anderson, K.P. & Stevens, J.G. (1988 b). The herpes simplex virus latency-associated transcript is spliced during the latent phase of infection. *Journal of Virology* **62**, 4577-4585.

Waldman, W.J., Adams, P.W., Orosz, C.G. & Sedmak, D.D. (1992). T lymphocyte activation by cytomegalovirus-infected, allogenic cultured human endothelial cells. *Transplantation* **54**, 887-896.

Walz, M.A., Price, R.W. & Notkins, A.L. (1974). Latent ganglionic infections with herpes simplex virus types 1 and 2: viral reactivation *in vivo* after neurectomy. *Science* **184**, 1185-1187.

Ward, P.L. & Roizman, B. (1994). Herpes simplex genes: the blueprint of a successful human pathogen. *Trends in Genetics* **10**, 267-274.

Warren, K.G., Devlin, M., Gilden, D.H., Wroblewska, Z., Brown, S.M., Subak-Sharpe, J. & Kuprowski, I. (1977). Isolation of herpes simplex virus from human trigeminal ganglia, including from one patient with multiple sclerosis. *Lancet* **2**, 637-639.

Warren, K.G., Brown, S.M., Wroblewska, Z., Gilden, D., Koprowski, H. & Subak-Sharpe, J. (1978). Isolation of latent herpes simplex virus from the superior cervical and vagus ganglions of human beings. *The New England Journal of Medicine* **298**, 1068-1069.

Warren, A.P., Ducroq, D.H., Lehner, P.J. & Borysiewicz, L.K. (1994). Human cytomegalovirus-infected cells have unstable assembly of major histocompatibility complex class I complexes and are resistant to lysis by cytotoxic T lymphocytes. *Journal of Virology* **68**, 2822-2829.

Warwick, R. & Williams, P.L. (1973). Grays anatomy, 35th ed. Edinburgh: Longman.

Watson, R.J. & Clements, J.B. (1978). Characterization of transcription-deficient temperature sensitive mutants of herpes simplex virus type 1. *Virology* **91**, 364-379.

Watson, K., Stevens, J.G., Cook, M.L. & Subak-Sharpe, J.H. (1980). Latency competence of thirteen HSV-1 temperature-sensitive mutants. *Journal of General Virology* **49**, 149-159.

Weber, S., Traunecker, A., Oliveri, F., Gerhard, W. & Karjalainen, K. (1992). Specific low-affinity recognition of major histocompatibility complex plus peptide by soluble T-cell receptor. *Nature* **356**, 793-796.

Wechsler, S.L., Nesburn, A.B., Watson, R., Slanina, S.M. & Ghiasi, H. (1988). Fine mapping of the latency-related gene of herpes simplex virus type 1: alternative splicing produces distinct latency-related RNAs containing open reading frames. *Journal of Virology* **62**, 4051-4058.

Wechsler, S.L., Nesburn, A.B., Zwaagstra, J. & Ghiasi, H. (1989). Sequence of the latency-related gene of herpes simplex virus type 1. *Virology* **168**, 168-172.

Wei, M.L. & Cresswell, P. (1992). HLA-A2 molecules in an antigen processing mutant cell contain signal sequence-derived peptides. *Nature* **356**, 443-446.

Weinheimer, S.P., Boyd, B.A., Durham, S.K., Resnick, J.L. & O'Boyle, D.R.II. (1992). Deletion of the VP16 open reading frame of herpes simplex virus type 1. *Journal of Virology* **66**, 258-269.

Weinstein, D.L., Walker, D. G., Akiyama, H. & McGeer, P. L. (1990). Herpes simplex virus type 1 infections of the CNS induces major histocompatibility complex antigen expression on rat microglia. *Journal of Neuroscience Research* **26**, 55-65.

Weissman, A.M., Bonifacino, J.S., Klausner, R.D., Samelson, L.E. & O'Shea, J.J. (1989). T cell antigen receptor: structure, assembly and function. *Year Immunology* **4**: 74-93.

Weller, S.K., Spadoro, A., Schaffer, J.E., Murray, A.W., Maxam, A.M. & Schaffer, P.A. (1985). Cloning, sequencing, and functional analysis of oriL, a herpes simplex virus type 1 origin of DNA synthesis. *Molecular and Cellular Biology* **5**, 930-942.

Wheeler, C.E. Jr. & Cabraniss, W.H. Jr. (1965). Epidemic cutaneous herpes simplex in wrestlers (herpes gladiatorum). *The Journal of the American Medical Association* **194**, 993-997.

Whitby, A., Blyth, W.A. & Hill, T.J. (1988). The effect of DNA hypomethylating agents on the reactivation of herpes simplex virus from latently infected mouse ganglia *in vitro*. *Archives of Virology* **97**, 117-144.

White, L.A., Keane, R.W. & Whittemore, S.K. (1994). Differentiation of an immortalized CNS neuronal cell line decreases their susceptibility to cytotoxic T cell lysis *in vitro*. *Journal of Neuroimmunology* **49**, 135-143.

Whitley, R.J., Lakeman, A.D., Nahmias, A. & Roizman, B. (1982). DNA restriction enzyme analysis of herpes simplex virus isolates obtained from patients with encephalitis. *New England Journal of Medicine* **307**, 1060-1062.

Whitley, R.J., Levin, M., Barton N., Hershey, B.J., Davis, G., Ronald, E., Keeney, J.W., Whelchel, J., Diethelm, G., Kartus, P. & Soong, S.J. (1984). Infections caused by herpes simplex virus in the immunocompromised host: natural history and topical acyclovir therapy. *Journal of Infectious Diseases* **150**, 323-329.

Whitley, R.J. (1990). Herpes simplex viruses. In *Virology*, pp. 1843-1887, 2nd Ed. Edited by Fields, B.N., Knipe, D.M., et al. New York: Raven Press.

Whitton, J.L. & Oldstone, M.B.A. (1989). Class I MHC can present an endogenous peptide to cytotoxic T lymphocytes. *Journal of Experimental Medicine* **170**, 1033-1038.

Wildy, P., Russell, W.C. & Horne, R.W. (1960). The morphology of herpes virus. *Virology* **12**, 204-222.

Wildy, P. (1967). The progression of herpes simplex virus to the central nervous system of the mouse. *Journal of Hygiene* **65**, 173-192.

Wildy, P., Field, H.J. & Nash, A.A. (1982). Classical herpes latency revisited. In: *Virus Persistence Symposium 33*, pp. 133-167 Edited by Mahy, B.W.J., Minson, A.C. & Darby, G.K. Cambridge: Cambridge University Press.

Williams, K., Hart, D., Fabre, J. & Morris, P. (1980). Distribution and quantitation of HLA-ABC and DR (Ia) antigens on human kidneys and other tissues. *Transplantation* 29, 274-279.

Williams, D.B., Swiedler, S.J. & Hart, G.W. (1985). Intracellular transport of membrane glycoproteins: Two closely related histocompatibility antigens differ in their rates of transit to the cell surface. *Journal of Cell Biology* **101**, 725-734.

Williams, D.B., Borriello, F., Zeff, R.A. & Nathenson, S.G. (1988). Intracellular transport of class I histocompatibility molecules. Influence of protein folding on transport to the cell surface. *Journal of Biological Chemistry* **263**, 4549-4560.

Williams, D.B., Barber, B.H., Flavell, R.A. & Allen, H. (1989). Role of β_2-microglobulin in the intracellular transport and surface expression of murine class I histocompatibilty molecules. *Journal of Immunology* **142**, 2796-2806.

Winoto, A., Steinmetz, M. & Hood, L. (1983). Genetic mapping in the major histocompatibility complex by restriction enzyme site polymorphisms: most mouse class I genes map to the Tla complex. *Proceedings of the National Academy of Sciences, USA* **80**, 3425-3429.

Wittels, M. & Spear, P.G. (1991). Penetration of cells by herpes simplex virus does not require a low pH-dependent endocytic pathway. *Virus Research* **18**, 271-290.

Wold, W.S.M. & Gooding, L.R. (1991). Region E3 of adenovirus: a cassette of genes involved in host immune surveillance and virus-cell interactions. *Virology* **184**, 1-8.

Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., Battye, F. & Schrader, J.W. (1984). Inducible expression of H-2 and Ia antigens on brain cells. *Nature* **310**, 688-691.

Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., McKimm-Breschkin, J.L. & Schrader, J.W. (1985). Interferon-γ induces the expression of H-2 and Ia antigens on brain cells. *Journal of Neuroimmunology* 7, 255-278.

Wong, G.H. & Goeddel, D.V. (1986). Tumour necrosis factor α and β inhibit virus replication and synergize with interferons. *Nature* **323**, 819-822.

Wooley, P.D. & Kudesia, G. (1990). Incidence of herpes simplex virus type-1 and type-2 from patients with primary (first-attack) genital herpes in Sheffield. *International Journal of STD and AIDS* **1**, 184-186.

Wright, H.T. (1973). Cytomegaloviruses. In: *The Herpesviruses*, pp. 353-388. Edited by Kaplan, A.S. New York: Academic Press.

Wroblewska, Z., Savage, K., Spivak, J.G. & Fraser, N.W. (1989). Detection of HSV-1 proteins prior to the appearance of infectious virus in mouse trigeminal ganglia during reactivation of latent infection. *Virus Research* **14**, 95-106.

Wu, C.A., Nelson, N.J., McGeoch, D.J. & Challberg, M.D. (1988). Identification of herpes simplex virus type 1 genes required for origin-dependent DNA synthesis. *Journal of Virology* **62**, 435-443.

Wudunn, D. & Spear, P.G. (1989). Initial interaction of herpes simplex virus with cells is binding to heparin sulfate. *Journal of Virology* **63**, 52-58.

Xiao, P. & Capone, J.P. (1990). A cellular factor binds to the herpes simplex virus type 1 transactivator Vmw65-dependent protein-DNA complex assembly with Oct-1. *Molecular and Cellular Biology* **10**, 4974-4977.

Yamamoto, T., Osaki, T., Yoneda, K. & Ueta, E. (1993). Immunological investigation of adult patients with primary herpes simplex virus-1 infection. *Journal of Oral Pathology and Medicine* **22**, 263-267.

Yamashita, Y., Shimokata, S., Mizuno, S., Yamaguchi, H. & Nishiyama, Y. (1993). Down-regulation of the surface expression of class I MHC antigens by human cytomegalovirus. *Virology* **193**, 717-736.

Yamashita, Y., Shimokata, S., Saga, S., Mizuno, S., Tsurumi, T. & Nishiyama, Y. (1994). Rapid degradation of the heavy chain of class I major histocompatibility complex antigens in the endoplasmic reticulum of human cytomegalovirus-infected cells. *Journal of Virology* **68**, 7933-7943.

Yang, Y., Waters, J.B., Fruh, K. & Peterson, P.A. (1992 a). Proteasomes are regulated by Interferon γ: Implications for antigen processing. *Proceedings of the National Academy of Sciences, USA* **89**, 4928-4932.

Yang, Y., Fruh, K., Chambers, J., Waters, J.B., Wu, L., Spies, T. & Peterson, P.A. (1992 b). Major histocompatibility complex (MHC)-encoded Ham 2 is necessary for antigenic peptide loading onto class I MHC molecules. *Journal of Biological Chemistry* **267**, 11669-11672.

Yannelli, J.R., Sullivan, J.A., Mandell, G.L. & Engelhard, V.H. (1986). Reorientation and fusion of cytotoxic T lymphocyte granules after interaction with target cells as determined by high resolution cinemicrography. *Journal of Immunology* **136**, 377-382.

Yasukawa, M. & Zarling, J.M. (1984). Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. II. Bifunctional clones with cytotoxic and virus-induced proliferative activities exhibit herpes simplex virus types 1 and 2 specific or type common reactivities. *Journal of Immunology* **133**, 2737-2742.

Yeh, L. & Schaffer, P.A. (1993). A novel class of transcripts expressed with late kinetics in the absence of ICP4 spans the junction between the long and short segments of the herpes simplex virus type 1 genome. *Journal of Virology* **67**, 7373-7382.

Yewdell, J., Lepham, C., Bacik, I., Spies, T. & Bennik, J. (1994). MHC-encoded proteasome subunits LMP2 and LMP7 are not required for efficient antigen presentation. *Journal of Immunology* **152**, 1163-1170.

Yokoyama, K. & Nathenson, S.G. (1983). Intramolecular organization of class I H-2 MHC antigens; localization of the allogenic determinants and the β_2m binding site to different regions of the H-2K^b glycoprotein. *Journal of Immunology* **130**, 1419-1425.

York, I.A., Roop, C., Andrews, D.W., Riddell, S.R., Graham, F.L. & Johnson, D.C. (1994). A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8$^+$ T lymphocytes. *Cell* **77**, 525-535.

Young, L.S. & Rowe, M. (1992). Epstein-Barr virus lymphomas and Hodgkin's disease. *Seminars in Cancer Biology* **3**, 273-284.

Youssoufian, H., Hammer, S.M., Hirsch, M.S. & Mulder, C. (1982). Methylation of the viral genome in an *in vitro* model of herpes simplex virus latency. *Proceedings of the National Academy of Sciences, USA* **79**, 2207-2210.

Zhang, X., Efstathiou, S. & Simmons, A. (1994). Identification of novel herpes simplex virus replicative intermediates by field inversion gel electrophoresis: implications for viral DNA amplification strategies. *Virology* **202**, 530-539.

Zhou, X., Momburg, F., Liu, T., Abdel Motal, U.M., Jondal, M., Hammerling, G.J. & Ljunggren, H.-G. (1994). Presentation of viral antigens restricted by H-2K^b, D^b or K^d in proteasome subunit LMP2- and LMP7-deficient cells. *European Journal of Imunology* **24**, 1863-1868.

Zinkernagel, R. & Doherty, P. (1974). Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. *Nature* **251**, 547-549.

Zwaagstra, J.C., Ghiasi, H., Nesburn, A.B. & Wechsler, S.L. (1989) *In vitro* promoter activity associated with the latency-associated transcript gene of herpes simplex virus type 1. *Journal of General Virology* **70**, 2163-2169.

Zwaagstra, J.C., Ghiasi, H., Slanina, S.M., Nesburn, A.M., Wheatley, S.C., Lillycrop, K., Wood, J., Latchman, D.S., Patel, K. & Wechsler, S.L. (1990). Activity of herpes simplex virus type 1 latency-associated transcript (LAT) promoter in neuron-derived cells: evidence for neuron specificity and for a large LAT transcript. *Journal of Virology* **64**, 5019-5028.

Zwaagstra, J.C., Ghiasi, H., Nesburn, A.B. & Wechsler, S.L. (1991). Identification of a major regulatory sequence in the latency associated transcript (LAT) promoter of herpes simplex virus type 1 (HSV-1). *Virology* **182**, 287-297.

Zweerink, H.J., Gammon, M.C., Utz, U., Sauma, S.Y., Harrer, T., Hawkins, J.C., Johnson, R.P., Sirotina, A., Hermes, J.D., Walker, B.D. & Biddison, W.E. (1993). Presentation of endogenous peptides to MHC class I-restricted cytotoxic T lymphocytes in transport deletion mutants T2 cells. *Journal of Immunology* **150**, 1763-1771.