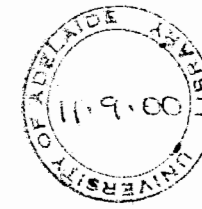

Myeloid Antigen Presenting Cell Populations in the Murine Uterus

Sarah Hudson (B Med Sci)

Department of Obstetrics and Gynaecology,
University of Adelaide, Adelaide, Australia

A thesis submitted to the University of Adelaide
in fulfillment of the requirements for admission to
the degree of Doctor of Philosophy

March 2000

Abstract

The uterus is unique amongst mucosal organs in that as well as generating protective immunity against pathogens, it must respond immunologically to antigens present in semen and on the conceptus in a manner which will allow pregnancy to ensue. Emerging evidence in other mucosal tissues indicates the capacity to discriminate between and respond appropriately to antigens of different types rests primarily with resident antigen presenting cells (APCs). Although the uterus is known to contain populations of myeloid APCs including macrophages (Mϕs) and cells with some features characteristic of dendritic cells, the precise phenotypes and functional potential of those cells to initiate immune responses have not been characterised.

The purpose of this study was therefore to undertake a detailed characterisation of the molecular phenotypes of the various APC populations present in the uterus, with a particular focus on those cells present at estrus, following insemination and at the time of embryo implantation (day 4 of pregnancy).

The first aim was to identify whether the APCs present in the murine uterus express cell surface phenotypic markers known to be associated with the events of antigen processing and presentation in other populations of interstitial Mϕs and dendritic cells. By immunohistochemistry, it was shown that cells with a dendriform morphology typical of APCs are present in the uteri of cycling virgin mice, and that these cells express F4/80 antigen, class A scavenger receptor, macrosialin, sialoadhesin, Ia and B7-2 at different relative abundancies.

The development of methods for dual-colour flow cytometric analysis of uterine cells established that at least three lineages of APCs are present within the uteri of estrous mice. On the basis of their side and forward scatter profiles and their cell surface phenotypes, the cells were designated 'undifferentiated Mϕs', 'differentiated Mϕs' and 'dendritic cells'. Both differentiated Mϕs and dendritic cells had a cell membrane profile consistent with their participation in antigen uptake, processing and presentation in the non-pregnant uterus. It was postulated that the undifferentiated Mϕs, which had a relatively simple cell surface phenotype, represented a precursor cell for activated Mϕs and possibly also dendritic cells.

That the majority of uterine APCs were sensitive in morphology and in their location within the endometrial stroma to ovarian steroid hormones was suggested by additional immunohisto-chemical and flow cytometric analysis of uteri from diestrous and ovariectomised mice. Notably, after ovariectomy the cells expressing each phenotypic marker were diminished in number, although a small population of APCs with a distinct, dendriform morphology was still evident. These cells were identified as dendritic cells, since the majority expressed Ia and

macrosialin very intensely, some expressed sialoadhesin and F4/80 but very few expressed scavenger receptor or B7-2.

The cytokine and leukocytic response of the murine uterus to mating is reminiscent of a classical inflammatory response. Upon exposure to seminal plasma, uterine epithelial cells secrete a diverse array of pro-inflammatory cytokines and chemokines, most notably GM-CSF, which is known to target myeloid leukocytes, affecting their recruitment and activation status. In accordance with this, the studies presented here show that in addition to Mϕs expressing F4/80 antigen, APCs expressing macrosialin, class A scavenger receptors, sialoadhesin, and B7-2 are all recruited into the day 1 pregnant uterus, where they accumulate in the superficial endometrial tissue in close proximity to luminal epithelial cells. Dual colour flow cytometry showed that mating affected the expression of activation markers by the APCs, and evidence of a more naïve phenotype in undifferentiated Mϕs suggested an accumulation of recently recruited cells. Cells expressing antigen presentation molecules Ia and CD1 were also present on day 1. Unexpectedly, a possible role for uterine epithelial cells in processing and presenting antigens within the uterine milieu was also evident since they too expressed macrosialin and CD1 on day 1 of pregnancy.

An examination of the APCs resident within the uteri of genetically GM-CSF-deficient mice suggested that although the recruitment of $F4/80^+$ Mϕs can occur normally in the absence of GM-CSF, this cytokine was requisite for normal activation and/or trafficking of Mϕs and dendritic cells within the uterine compartments before and after insemination. Most notably, differentiated APCs from GM-CSF-deficient mice were reduced in number and the intensity of their expression of activation markers was relatively low.

APCs contained within the day 4 pregnant uterus were found to be significantly reduced in number compared to day 1 of pregnancy, and were preferentially located in the deep endometrial tissue. A clustering of APCs in a formation reminiscent of organised lymphoid tissue was also noted in the deep endometrium of many uteri at day 4 of pregnancy, although the exact cellular composition of these structures was not determined. FACS analysis suggested that the majority of the APCs that were retained within uteri of day 4 pregnant mice were highly differentiated, since they expressed all of the activation markers at relatively high levels.

In order to further investigate the phenotype of uterine Mϕs by *in vitro* analysis, $F4/80^+$ cells were purified with the use of immunomagnetic cell selection techniques from single cell suspensions released by enzymatic digestion of uteri. These cells exhibited features common to most *ex vivo* Mϕ populations, being adherent to tissue-culture grade plastic and rapidly phagocytic of small latex beads. When assessed for their immunoaccessory function in a spleen cell mitogenesis assay, the Mϕs were found to be potently immunosuppressive due to the

synthesis of a soluble inhibitory molecule which was determined not to be a prostaglandin nor nitric oxide. The immunoinhibitory phenotype of uterine Mϕs was found to be most potent at estrus and day 4 of pregnancy, but was moderated after insemination, and particularly after ovariectomy when the Mϕs were found to be immunostimulatory in nature. Thus a role for steroid hormone- and insemination-regulated cytokines in regulating the secretory phenotype of uterine Mϕs seems likely. However, the highly complex nature of the regulation of uterine Mϕs was illustrated by experiments showing that this inhibitory phenotype could not be induced by steroid hormone-replacement of ovariectomised mice, nor could it be removed by culture in GM-CSF.

In summary, these studies suggest that myeloid APCs present within the murine uterus are similar to APC populations found in other mucosal tissues such as the lung, where they play a role in mediating tissue remodeling, immune homeostasis and the initiation of antigen-specific immunity. During the estrous cycle, the recruitment and *in situ* differentiation of uterine Mϕs and dendritic cells appears to be regulated in an ovarian steroid hormone-dependent manner, presumably as a result of the release of cytokines from uterine stromal and/or epithelial cells. The post-mating recruitment into the endometrium of increased numbers of APCs expressing molecules suggestive of an activated phenotype indicates an enhanced capacity for antigen uptake and processing at this time of exposure to paternal and other antigens. The pleiotrophic cytokine GM-CSF seems particularly important in mediating the activation of uterine Mϕs and particularly dendritic cells at day 1. By the fourth day of pregnancy, the uterine APCs are markedly reduced in number, are of a relatively differentiated phenotype and exhibit an altered pattern of distribution in the tissue.

To conclude, these studies have identified and characterised abundant and heterogeneous populations of Mϕs and dendritic cells within uterine tissues which express a large number of molecules known to be associated with antigen processing and presentation. The maturation and activation of these cells appears to be acutely responsive to the diverse microenvironments induced in the uterus by ovarian steroid hormones and the events of early pregnancy. The precise roles of APCs within the uterine milieu are yet to be identified. However, based on the current study and other reports of mucosal organs some speculations can be made. The initiation of antigen-specific immunity by uterine APCs would be vital in the generation of protective immunity against opportunistic pathogens. However, in order to accommodate insemination and pregnancy, the APCs would also need to be able to generate immune responses which mediate tolerance of innocuous antigens encountered in semen and on the semi-allogeneic conceptus. The mechanisms underpinning such a diverse array of immune responses to antigens encountered within the uterus are yet to be elucidated, but would almost certainly be determined principally

by cytokine-regulated events of antigen uptake, processing and presentation by uterine Mϕs, dendritic cells and perhaps even epithelial cells. Future studies directed at a more detailed analysis of the cell membrane and secretory phenotypes of uterine APCs in the uterus and its draining lymph nodes will address these issues.

Table of Contents