Regulation of chemokine receptor expression and function on $CD4^+$ T lymphocytes during central nervous system inflammation

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Abstract

Chemokines are a family of cytokines that exhibit selective chemoattractant properties for target leukocytes, including $CD4^+$ T lymphocytes, and play a significant role leukocyte migration. However, a target leukocyte can only respond to a chemokine if it expresses the cognate receptor(s). Recent studies have demonstrated alterations in both chemokine and chemokine receptor expression patterns in the CNS during experimental autoimmune encephalomyelitis (EAE), a model for Multiple Sclerosis. Accordingly, the aim of the research presented in this thesis was to investigate chemokine receptor regulation and function on $CD4^+$ T lymphocytes during T cell-mediated central nervous system (CNS) inflammation *in vivo*. In the proteolipid protein (PLP)-induced model of EAE, two inflammatory (CXCR3 and CCR5) and one supposedly homeostatic (CXCR4) chemokine receptors were upregulated on $CD4^+$ T cells during antigen-dependent clonal selection in the draining lymph nodes. As the $CD4^+$ T cells migrated through the blood and into the CNS tissue, expression of these receptors remained elevated such that, at the peak of clinical disease, the majority of neuroantigen-specific $CD4^+$ T cells in the CNS expressed elevated levels of CXCR4, CXCR3 and CCR5. Detailed characterisation of these receptors revealed that upregulation occurred in co-ordination with cellular division.

Subsequent experiments were performed in order to determine the biological consequences of specific chemokine/receptor interactions during EAE. Amino terminal modifications of chemokines, which convert agonists to antagonists, have previously been shown to interfere with ligand/receptor interactions during inflammation. Therefore, a series of synthetic N-terminal chemokine mutants were initially tested *in vitro* for their ability to act as antagonists in preventing the migration of neuroantigen-activated lymphocytes to ligands of the receptors CXCR4, CXCR3 and CCR5. These analyses revealed that the synthetic mutants SDF-1 P2G, I-TAC 4-79 and RANTES 9-68 possessed potent antagonistic capacities. Following EAE induction, treatment every second day with the antagonists until day 15 resulted in a significant decrease in the severity of the neurological symptoms of EAE. Histological analyses demonstrated that the reduction in disease severity corresponded with a reduced number of inflammatory infiltrates in the spinal cords of antagonist-treated mice at peak clinical disease compared with control-treated mice.

The ability to separate the disease process into two separate phases (sensitisation and effector) using adoptive transfer experiments provided a means to investigate the temporal and spatial control that specific chemokine/receptor interactions exerted during the pathogenesis of EAE. Accordingly, a series of *ex vivo* proliferation assays and adoptive transfer experiments were conducted. From these experiments, a potential role for the SDF-1/CXCR4 interaction was identified in the sensistisation phase of the disease. These results indicated that SDF-1/CXCL12 and CXCR4 interactions not only play a role in homeostasis, but may also provide costimulatory signals to antigen-stimulated $CD4^+$ T cells. Converely, roles for I-TAC/CXCR3 and RANTES/CCR5 interactions, but not SDF-1/CXCR4 interactions were identified in the effector phase of EAE. Collectively, the results generated in the present thesis, together with those from other studies, enabled the construction of a model detailing the temporal and spatial parameters of chemokine/chemokine receptor regulation of $CD4^+$ T cell activation and migration during a $CD4^+$ T cell-mediated immune response in the CNS.

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