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Chemokines in chronic progressive neurological diseases: HTLV-1 associated myelopathy and multiple sclerosis

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It is hypothesized that in MS and HTLV-1, chemokine and chemokine receptor expression are important mechanisms by which T cells migrate to sites of inflammation. Preliminary evidence supports the roles of several chemokines, including MIP 1β, in mediating the enhanced migration capacity of MS derived PBLs. In addition, the ligand CCR-5 seems to be up regulated on PBLs from some MS patients. Analysis of T cell clones does not reveal a definite correlation between cytokine phenotype and chemokine receptor profile. The chemokines and chemokine receptor family are likely to be important molecules in chronic progressive neurological diseases, in which immune cells invade the central nervous system.

Keywords: multiple sclerosis; HTLV-1; chemokine receptors; migration

Introduction

Chemokines are 8–12 kd chemoattractant cytokines characterized by a four cysteine motif (Luster, 1998; Ransohoff *et al*, 1996; Schall, 1994; Taub, 1996). The presence or absence of an intervening amino acid(s) (X) between the N terminal cysteines defines the CXC/CX_3C and CC families, respectively. A third family, C chemokines, lacks an N terminal cysteine but is otherwise homologous. It was originally thought that the CXC chemokines mediated neutrophil migration and CC chemokines mediated mononuclear cell migration, but now a subclass of CXC chemokines that lacks the sequence gluatamic acid–leucine–arginine near the N terminal are potent chemotactic agents for activated T cells. Chemokines enhance chemoattraction and migration by several pathways (Taub, 1996). Chemokines bind to chemokine receptors, which are members of the seven transmembrane receptor family and are coupled to G proteins. Chemokine signaling induces a conformational change in integrin molecules and enhances their binding to the cell adhesion molecules ICAM and VCAM. In addition, chemokines may have direct effects on T cell activation and release of matrix metalloproteinases that digest the extracellular matrix underlying cerebral endothelium. The chemokines and chemokine receptor family are therefore likely to be important molecules in chronic progressive neurological diseases in which immune cells invade the central nervous system.

Multiple sclerosis is a chronic, predominantly demyelinating, disease of the central nervous system, characterized by perivenular inflammatory cell infiltrates (Martin and McFarland, 1995; Raine, 1994). Although the pathogenesis of the disease is unknown, a number of mechanisms have been suggested, including: (1) the disease is associated with autoreactive T cells directed against myelin peptides or (2) the disease is secondary to an inappropriate immune response to microbial antigens, predominantly viral. The concepts are not mutually exclusive, since an immune response directed against a virus may result in inappropriate immune reactivity through several mechanisms including persistent viral infection with: (1) molecular mimicry between viral epitopes and myelin or other CNS constituents; (2) inflammatory cytokine mediated bystander damage, or (3) viral infection of oligodendrocytes that leads to impaired myelin

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maintenance and an indirect immune response to damaged tissues.

Several lines of evidence support the autoimmune theory including: (1) immunogenetic predisposition conveyed by certain HLA DR molecules; (2) a higher prevalence in women (3 : 1) as is seen in other autoimmune diseases; (3) a greater frequency of autoreactive T cells in MS patients' peripheral blood that do not require a second or costimulatory signal, which suggests they have been primed *in vivo* (Lovett-Racke *et al*, 1998), and (4) the clinical response in some patients to immunosuppressive therapies (Lucchinetti and Rodriguez, 1997; Martin and McFarland, 1995). Proponents of the autoimmune hypothesis also suggest that intercurrent viral or bacterial infections may activate autoreactive T cells, which may be associated with disease exacerbations.

Epidemiological studies suggest there may be a critical environmental trigger that predisposes people to getting MS who spend their childhood years in temperate zones, especially areas around 45° latitude (Hogancamp *et al*, 1997). In addition, studies of monozygotic twins have demonstrated a 30–40% concordance rate, which again argues that there must be an environmental factor that provokes the process in those individuals who are immunogenetically predisposed (McFarland *et al*, 1985). Numerous viruses have been implicated in the pathogenesis of MS over the years. Reports of elevated titers of EBV, measles virus, canine distemper virus, HTLV-1 and recently HHV-6 have at times raised hopes that one or more of these viruses may be implicated in the pathogenesis of the disease (Lucchinetti and Rodriguez, 1997; Soldan *et al*, 1997). HTLV-1 has since been demonstrated to be the etiologic agent for Tropical Spastic Paraparesis-HTLV-1 associated myelopathy (HAM-TSP), a chronic progressive neurological disorder with clinical features similar to some forms of MS (Hollberg and Hafler, 1993; Nakagawa *et al*, 1995). With respect to the other viruses, some critics argue that there is non-specifically enhanced immune reactivity in MS that accounts for elevated anti-viral antibody titers. Recently, our lab has found evidence in some MS patients for an IgM response to HHV-6, which is the cause of Roseola and a ubiquitous childhood infection (Caserta and Breese Hall, 1993). In addition, we are able to amplify HHV-6 DNA from the serum of approximately one-third of MS patients (Soldan *et al*, 1997). Others have found evidence for HHV-6 expression in the oligodendrocytes of MS patients (Challoner, 1995). Whether these data are an artifact of inflamed tissue and enhanced immunoreactivity is unclear, but the role of herpes viruses remains of particular interest because of their propensity to encode chemokines and chemokine receptors (Meini *et al*, 1996; Smith *et al*, 1996).

Viruses and chemokines

Many of the large double stranded DNA viruses are now recognized to contain genes that encode proteins, which allow the virus to evade the host immune response. Viral homologues of IL-10, IFN-γ and TNF receptor, IFN responsive elements (IRE), apoptosis inhibiting proteins (BCL-2) and chemokines and chemokine receptors have been identified (Smith *et al*, 1996). HHV-6 has regions in its genome which encode for two chemokines and a chemokine receptor (Gompels *et al*, 1995). HHV-8 also has high homology for chemokines and has recently been implicated in the pathogenesis of Kaposi's sarcoma and certain CNS lymphomas (Ensoli and Sturzl, 1998). CMV also has regions of homology with chemokines and chemokine receptors. The chemoattractant molecules may be an important mechanism by which lymphotropic herpes viruses evade the initial immune response and propagate themselves to immunologically privileged tissues. According to this hypothesis, viral infected lymphocytes may express chemokines or chemokine receptors, which allow them to preferentially migrate to the brain. Viral infection of lymphocytes may occur as the primary event with subsequent migration to CNS tissues, or the lymphocytic inflammatory reaction may occur secondarily in response to a primary CNS infection. Astrocytes and endothelial cells can produce chemokines during inflammatory situations or in response to infection and may signal inflammatory cells (Ransohoff *et al*, 1996; Ebnet *et al*, 1997; Gupta *et al*, 1998).

The mechanisms by which HIV-1 infection can cause CNS pathology are beyond the scope of this article, but may be determined to some extent by chemokine or chemokine receptor expression. It is clear that the chemokine receptors CCR-5 and CXCR-4 are necessary cofactors for the infection of T cells by the macrophage tropic and lymphotropic strains respectively of the virus, and that enhanced expression of certain chemokines may be protective by causing receptor down regulation (Dragic *et al*, 1996; Kelly *et al*, 1998). The CCR-5 mutation that protects against HIV-1 infection in a minority of Caucasians does not seem to offer any protection against MS (Bennetts *et al*, 1997).

HTLV-1

HTLV-1 infection is endemic in certain regions of the Caribbean and only a minority of the infected people manifest medical illness (Hollberg and Hafler, 1993; Nakagawa *et al*, 1995). Disorders that have been definitively associated with HTLV-1 infection include adult T cell leukemia (ATL) and HAM-TSP, a chronic progressive myelopathy with features similar to primary progressive MS.

ATL is characterized by massive monoclonal expansion and subsequent infiltration of infected circulating CD4+ lymphocytes into several tissues and secondary lymphoid organs. The extravasation of malignant cells into tissues involves the same adhesive pathways responsible for migration of inflammatory cells. ATL cells spontaneously adhere to endothelial cells, and this appears to be mediated by enhanced expression of the chemokines MIP-1 α and MIP-1 β (Tanaka *et al*, 1998). Chemokines bind to seven transmembrane G-protein-coupled receptors, which causes a conformational change in the integrin LFA-1 and appears to enhance firm adhesion to the ligand ICAM.

HAM-TSP is a neurological manifestatioan of HTLV-1 infection. Patients develop a slowly progressive paraparesis with minimal sensory involvement. Several inflammatory mediators including TNF, adhesion molecules, and MCP-1 are expressed by infiltrating cells (Fox *et al*, 1996; Umehara *et al*, 1996). HAM-TSP patients have very high frequencies of HTLV-1 specific CD8+ CTL and these have been shown to produce numerous inflammatory mediators including MIP-1 α and MIP-1 β (Biddison *et al*, 1997a). Also, human T cell lines infected with HTLV-1 express the chemokines SDF, RANTES, and MIP-1 α and MIP-1 β. Expression of the HTLV-1 viral protein Tax induces the expression of these chemokine genes as well as other inflammatory cytokines (Baba *et al*, 1996; Arai *et al*, 1998; Mendez *et al*, 1997). Therefore, it seems likely that the same mechanisms involved in leukocyte recruitment to inflammatory sites are involved in mediating tissue damage by HTLV-1 infected T cells.

It is also possible that HTLV-1 can infect CNS cells directly. HTLV-1 DNA has been detected by *in situ* hybridization in cells that appear to be astrocytes (Lehky *et al*, 1995). Astrocytes are an important source of inflammatory mediators including chemokines (Ransohoff *et al*, 1996). Therefore, HTLV-1 infection within the CNS may induce recruitment of antigen specific T cells through chemokine signaling pathways. This recruitment process may be very similar to what has already been elegantly shown to occur in ATL.

EAE

EAE is an experimental model of immune mediated demyelination, which has some features in common with MS (Martin and McFarland, 1995). The disease can be induced by inoculation of myelin reactive T cells that migrate to the CNS and mediate inflammatory destruction of myelin. Recent studies have demonstrated the expression of many chemokines including; RANTES, MIP-1 α, MIP-1 β, IP-10, MCP-1 and 3, KC, TCA3 in conjunction with inflammatory cell infiltrates, but before clinical signs of disease, which suggests these molecules play a critical role in the immune amplification process that leads to demyelination (Berman *et al*, 1996; Glabinski *et al*, 1997; Godiska *et al*, 1995). Infiltrating T cells seem to be primarily responsible for production of RANTES and MIP-1 α, MIP-1 β, whereas MCP-1, IP-10, and KC expression is confined to astrocytes (Glabinski *et al*, 1995, 1997). The precise roles of these molecules is currently being better defined and is discussed elsewhere.

Multiple sclerosis

As discussed previously, the etiology of MS remains unknown. The hypothesis that a microbial antigen triggers an aberrant immune response in certain immunogenetically predisposed patients remains attractive. However, it is possible that the disease is a result of a purely autoimmune process (Lucchinetti and Rodriguez, 1997). Regardless, the pathology demonstrates perivenular inflammatory cell infiltrates, and there seems to be a definite clinical response to immunomodulating therapies. Therefore, it is likely that in MS chemokine and chemokine receptor expression are important mechanisms by which antigen driven T cells migrate to sites of inflammation (Merrill and Benveniste, 1996; Merrill and Murphy, 1997; Ransohoff *et al*, 1996). An analysis of chemokines within MS brains confirmed expression of RANTES, MIP-1 α, MIP-1 β, and MCP-1. RANTES appeared to be restricted to perivascular sites, while MIP-1 α, MIP-1 β, and MCP-1 were also expressed by glial cells within and around plaques (Simpson *et al*, 1998). Both CD8+ and CD4+ myelin reactive T cell clones derived from peripheral blood may express chemokines including MIP-1 α, MIP-1 β, IP-10, and IL-16, and the matrix metalloproteinases MMP-9 and MMP-2 (Biddison *et al*, 1997b, 1998 and unpublished observations). PBMC's derived from MS patients have increased adherence in binding assays and increased migration across a fibronectin matrix in response to the chemokines RANTES, MIP-1 α, and MCP-1 (Stuve *et al*, 1997). Interferon beta, which is therapeutic in MS inhibits migration and may do so partly by abrogating MCP-1 induced production of the matrix degrading enzyme MMP-9 (Leppert *et al*, 1995; Stuve *et al*, 1996).

Results and Discussion

Our studies confirm modestly enhanced migration of MS patient PBLs as compared to controls, towards the chemokine MIP-1β (Figure 1a). There is minimal to no spontaneous migration from PBLs, but the addition of IL-2 and chemokines results in enhanced migration (Figure 1b). However, there is significant variability from one individual to another, which suggests that the expression of the

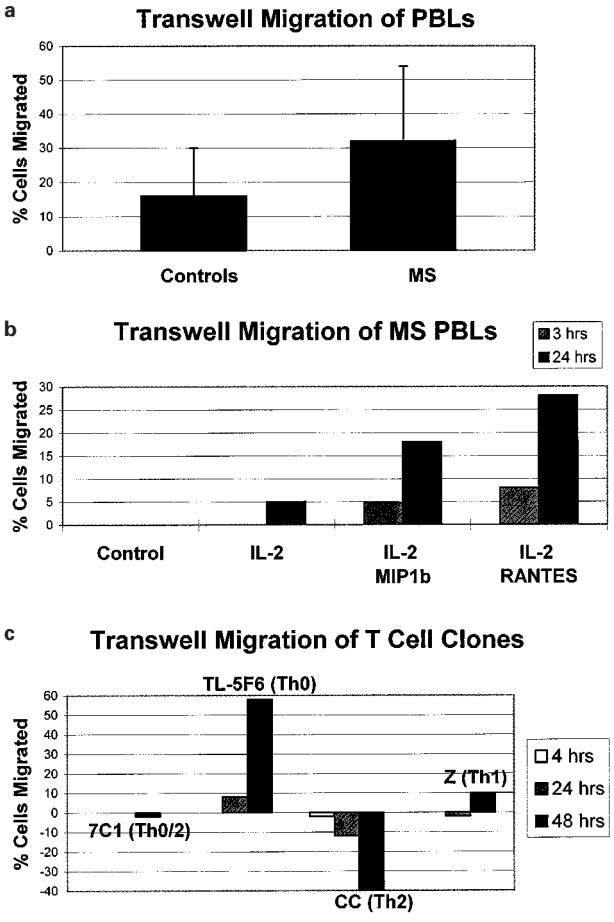

Figure 1 Transwell migrations of lymphocytes: (**a**) Comparison of PBL transwell migration (±s.d.) to MIP1β in ten controls and ten MS patients. (**b**) Transwell migrations of MS patient PBLs at 3 and 24 h comparing different chemoattractant conditions. (**c**) Transwell migrations of four different T cell clones (with different cytokine phenotypes) to MIP1β (% migration is normalized to migration without the chemokine thus negative % values indicate less migration than the control).

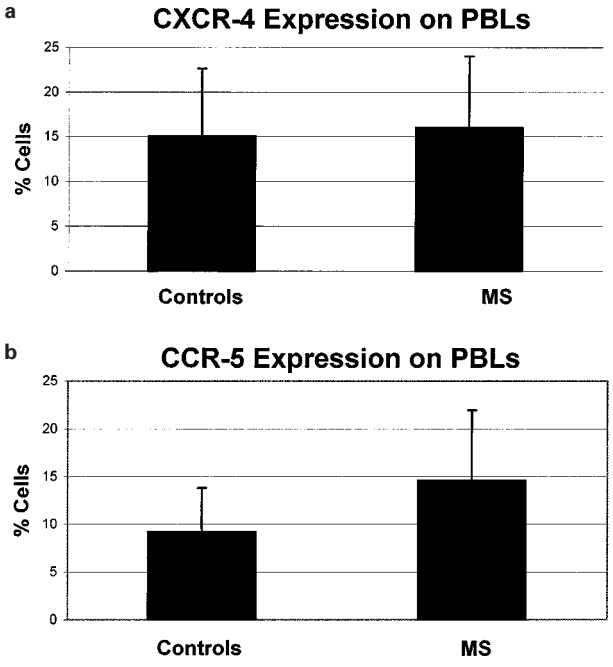

Figure 2 Flow cytometry of PBL chemokine receptors: (**a**) Mean (±s.d.) CXCR-4 expression on PBLs from ten control subjects and ten MS patients. (**b**) Mean CCR-5 expression on PBLs from the same cohort as in (**a**).

chemokine receptors must also be divergent. This has also been observed at the clonal level, and even within clones derived from the same patient (Figure 1c). Therefore, we undertook to determine whether chemokine receptor expression is selectively up regulated on MS PBLs or T cell clones with specific cytokine expression patterns.

Flow cytometric analysis of controls and MS patient PBLs revealed equivalent levels of CXCR-4, which is known to be constitutively expressed (Figure 2a). However, there appeared to be a significantly increased number of CCR-5 positive cells in certain MS patient samples (Figure 2b and c). This is of particular interest because CCR-5 expression was recently found to be enhanced in polarized Th1 cytokine producing lines *in vitro* (Sallusto *et al*, 1998). Several lines of evidence suggest that MS is mediated by Th1 producing cells, and therefore enhanced chemokine receptor expression could account for preferential migration of this subset to the CNS (Martin and McFarland, 1995; Rieckmann *et al*, 1995). At this time it is unclear whether the association between cytokine phenotype and chemokine receptors exists *in vivo*.

At the clonal level, we have found increased numbers of both CXCR-4 and CCR-5 positive cells as compared to PBLs. In addition, we examined the T cell clones for CCR-3 and CXCR-3 expression to determine if the chemokine receptor expression pattern correlated with the clones known cytokine expression pattern, as has been recently described in highly polarized T cell lines (Qin *et al*, 1998; Sallusto *et al*, 1998). All the clones had surface expression of all four chemokine receptors (Figure 3). Although chemokine receptor expression varied widely from clone to clone, there was no association between the Th2 clones and CCR-3 expression, or between Th1 and CXCR-3 or CCR-5 expression as has been found in the above mentioned studies that employed cell lines artificially driven towards separate ends of the cytokine spectrum (Figure 3a and b). This is in keeping with previous studies of the MBP clones that found not only significant heterogeneity in cytokine expression, but also very few purely Th1 or Th2 clones (Hemmer *et al*, 1996).

Migration chamber studies were done to assess the chemotactic propensity of the clones using the relevant CKR ligands; RANTES, MIP-1 β, IP-10, SDF-1, and eotaxin. We observed a greater tendency for the clones to migrate towards IP-10 > MIP-1

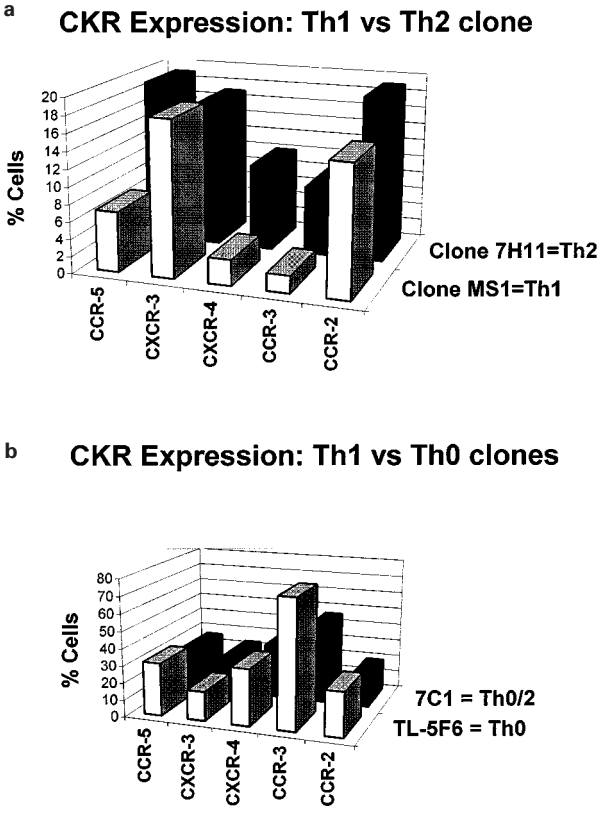

Figure 3 Flow cytometry of T cell clone chemokine receptors: (**a**) Mean chemokine receptor expression for clone 7H11 (Th2 cytokine phenotype (high IL-4 low IFN-γ by ELISA of conditioned medium)) and clone MS-1 (Th-1 cytokine phenotype). (**b**) Mean chemokine receptor expression for clone TL-5F6 (Th0 cytokine phenotype (moderate IL-4 and high IFN-γ by ELISA of conditioned medium)) and clone 7C1 (Th-0/2 high IL-4 and moderate IFN-γ cytokine phenotype).

$\beta >$ eotaxin (Figure 4). This again did not correlate well with cytokine or CKR expression, although fits with a slight TH1 predominance. The discrepancy between the CKR surface expression and functional capacity to migrate suggests that alternative mechanisms such as binding avidity and metalloproteinase production are involved in migration across the coated filters.

Materials and methods

Cells

Ten patients with clinically definite MS and ten normal controls were studied. PBMCs were isolated from heparinized whole blood (Calabresi *et al*, 1997) and allowed to adhere for 2 h to tissue culture wells. Four MBP reactive T cell clones with known cytokine phenotypes were also studied (Hemmer *et al*, 1996).

Migration

To better define the role of chemokines in MS, we have employed Transwell migration chambers

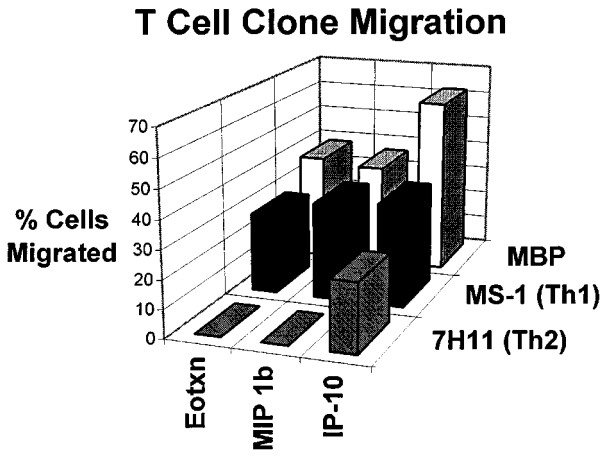

Figure 4 Cell clone migrations: percent cells migrated to chemokines divided by spontaneous migration on X axis, chemokines used for chemotactic gradient on Y axis, and T cell clones migrated on Z-axis.

(Costar) to study chemotaxis of MS patient PBLs as well as MBP reactive T cell clones. Briefly, 5×10^5 lymphocytes were placed in the upper chamber of a Transwell insert in IMDM supplemented with 10% human AB serum (Sigma) and 10 u/ml IL-2. Transmigration across a 5 mm pore polycarbonate filter coated with Matrigel (Becton-Dickinson) was assessed at the indicated time points by counting the cells from the lower chamber on a hemacytometer.

Flow cytometry

2×10^5 PBLs/T cell clones were placed on ice for 60 min in FACS buffer containing phosphate buffered saline (PBS) and 0.1% bovine serum albumin (BSA) and incubated with PE conjugated antibody to CD3 (Sigma) and monoclonal antibodies to human CCR5, CXCR-4, CXCR-3, CCR-3, and CCR-2 (R&D Systems and kind gift of Walter Newman, LeukoSite). A secondary FITC labeled antibody was incubated for 30 min. Appropriate isotype controls were also employed. The cells were washed and analyzed immediately on a FACSCAN (Becton-Dickinson, San Jose, CA, USA). Data are presented as % of cells staining positive for both CD3 and the respective chemokine receptor after subtracting out background from subtype control antibodies.

Conclusion

These data, in conjunction with previous studies that demonstrated chemokine production by MBP and PLP clones, indicate that chemokine-chemokine receptor signaling is likely an important mechanism by which antigen specific lymphocytes migrate to sites of inflammation and recruit

secondary immune cells. It remains unclear whether one component of this signaling pathway is preferentially up regulated in MS, either by a virus or as part of a Th1 immune bias. It is also uncertain what role astrocytes or endothelial cells might play in the recruitment process, as these cells can express chemokines and chemokine receptors.

It is likely that chemokines and chemokine receptors also are important in the pathogenesis of HAM/TSP. The role of chemokines in the HTLV-1 hematological disease, ATL, has already been demonstrated, and recent data suggest the HTLV-1 Tax can induce chemokine production. It is unclear whether immune cells, astrocytes, or both are infected, and if the virus does indeed initiate an immune response that ultimately results in tissue damage.

Our understanding of chemokines and chemokine receptors in immune processes has grown exponentially. Recent data suggest that these molecules also likely have significant roles in non-immune cells, both to signal the immune system to injury and as a part of neuronal migration during embryogenesis and possibly tissue repair (Zou *et al*, 1998). It is likely that our appreciation of the diverse roles of the chemokines in diseases of the nervous system will undergo a similar expansion.

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References

Arai M, Ohashi T, Tuskahara T, Murakami T, Hori T, Uchiyama T, Yamamoto N, Kannagi M, Fujii M (1998). Human T-cell leukemia virus type 1 TAx protein induces the expression of lymphocyte chemoattractant SDF-1/PBSF. *Virology* **241:** 298–303.

Baba M, Imai T, Yoshida T, Yoshie O (1996). Constitutive expression of various chemokine genes in human Th-0 cell lines infected with human T-cell leukemia virus type 1: role of the viral transactivator Tax. *Int J Cancer* **66:** 124–129.

Bennetts BH, Teutsch SM, Buhler MM, Heard RN, Stewart GJ (1997). The CCR-5 deletion mutation fails to protect against multiple sclerosis. *Human Immunol* **58:** 52–59.

Berman J, Guida M, Warren J, Amat J, Bronsan C (1996). Localization of monocyte chemoattractant peptide-1 expression in the central nervous system in experimental autoimmune encephalomyelitis and trauma in the rat. *J Immunol* **156:** 3017–3023.

Biddison W, Cruikshank W, Center D, Pelfrey C, Taub D, Turner R (1998). $CD8^+$ Myelin Peptide-Specific T cells can chemoattract $CD4^+$ Myelin Peptide-Specific T cells: Importance of IFN-Inducible Protein 10. *The J Immunol* **160:** 444–448.

Biddison WE, Kubota R, Kawanishi T, Taub DD, Cruikshank WW, Center DM, Connor EW, Utz U, Jacobson S (1997a). Human T cell leukemia virus type I (HTLV-I)-specific CD8+ CTL clones from patients with HTLV-I-associated neurologic disease secrete proinflammatory cytokines, chemokines, and matrix metalloproteinase. *J Immunol* **159:** 2018–2025.

Biddison WE, Taub DD, Cruikshank WW, Center DM, Connor EW, Honma K (1997b). Chemokine and matrix metalloproteinase secretion by myelin proteolipid protein-specific CD8+ T cells. *The J Immunol* **158:** 3046–3053.

Calabresi PA, Pelfrey CM, Tranquill LR, McFarland HF (1997). VLA-4 expression on peripheral blood lymphocytes is downregulated after treatment of multiple sclerosis with interferon beta. *Neurology* **49:** 1111–1116.

Caserta MT, Breese Hall C (1993). Human Herpes Virus-6. *Ann Rev Med* **44:** 377–383.

Challoner P, Smith KT, Parker JD, MacLeod DL, Coulter SN, Rose TM, Schulz J, Bennett JL, Garber RL, Chang M, Schad PA, Stewart PM, Nowinski RC, Brown JP, Burmer GC (1995). Plaque associated expression of human herpes virus 6 in multiple sclerosis. *Proc Natl Acad Sci* **92:** 7440–7444.

Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381:** 667–673.

Ebnet K, Brown K, Siebenlist U, Simon M, Shaw S (1997). Borrelia burgdorferi activates nuclear factor-κb and is a potent inducer of chemokine and adhesion molecule gene expression in endothelial cells and fibroblasts. *J Immunol* **158:** 3285–3292.

Ensoli B, Sturzl M (1998). Kaposi's Sarcoma: A result of the Interplay among Inflammatory Cytokines, Angiogenic Factors and Viral Agents. *Cytokine and Growth Factors Rev* **9:** 63–83.

Fox RJ, Levin MC, Jacobson S (1996). TNF alpha expression in the spinal cord of HAM/TSP patients. *J Neurovirol* **2:** 323–329.

Glabinski A, Tani M, Strieter R, Tuohy V, Ransohoff R (1997). Synchronous synthesis of alpha and beta chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. *Am J Pathol* **150:** 617–630.

Glabinski AR, Tani M, Tuohy VH, Tuthill RJ, Ransohoff RM (1995). Central nervous system chemokine mRNA accumulation follows initial leukocyte entry at the onset of acute murine experimental autoimmune encephalomyelitis. *Brain Behav Immunol* **9:** 315–330.

Godiska R, Chantry D, Dietsch GN, Gray PW (1995). Chemokine expression in murine experimental allergic encephalomyelitis. *J Neuroimmunol* **58:** 167–176.

Gompels U, Nicholas J, Lawrence G *et al* (1995). The DNA sequence of human herpes virus-6: structure, coding content, and genome evolution. *Virology* **209:** 29–51.

Gupta S, Lysko P, Pillarisetti K, Ohlstein E, Stadel J (1998). Chemokine Receptors in Human Endothelial Cells. *J Biol Chem* **273:** 4282–4287.

Hemmer B, Vergelli M, Calabresi PA, Huang T, McFarland H, Martin R (1996). Cytokine phenotype of human autoreactive T cell clones specific for the immunodominant myelin protein peptide. *J Neurol Sci* **45:** 852–862.

Hogancamp W, Rodriguez M, Weinshenker BG (1997). The Epidemiology of Multiple Sclerosis. *Mayo Clin Proc* **72:** 871–878.

Hollberg P, Hafler DA (1993). Pathogenesis of disease induced by HTLV-1 infection. *N Engl J Med* **328:** 1173–1182.

Kelly MD, Hassan MN, Adams SL, Cunningham AL, Lloyd AR (1998). Cutting Edge: Dichotomus Effects of β-Chemokines on HIV Replication in Monocytes and Monocyte-Derived Macrophages. *J Immunol* **160:** 3091–3095.

Lehky TJ, Fox CH, Levin MC, Flerlage N, Izumo S, Sato E, Raine CS, Osame M, Jacobson S (1995). Detection of HTLV-1Tax RNA in the CNS of HAM/TSP patients by in situ hybridization. *Ann Neurol* **47:** 167–175.

Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL (1995). T cell gelatinases mediate basement membrane transmigration in vitro. *J Immunol* **154:** 4379–4389.

Lovett-Racke AE, Trotter JL, Lauber J, Perrin PJ, June CH, Racke MK (1998). Decreased dependence of myelin basic protein-reactive T cells on CD28-mediated costimulation in multiple sclerosis patients: a marker of activated/memory T cells. *J Clin Inv* **101:** 725–730.

Lucchinetti CF, Rodriguez M (1997). The controversy surrounding the pathogenesis of the multiple sclerosis lesion. *Mayo Clin Proc* **72:** 665–678.

Luster AD (1998). Chemokines - Chemotactic Cytokines That Mediate Inflammation. *N Engl J Med* **338:** 436–445.

Martin R, McFarland HF (1995). Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. *Crit Rev Clin Lab Sci* **32:** 121–182.

McFarland HF, Greenstein F, McFarlin DE, *et al* (1985). Family and twin studies in multiple sclerosis. *Ann NY Acad Sci* **436:** 118–124.

Meini E, Fickenscher H, Fleckenstein B (1996). Chemokine receptors and chemokine-inducing molecules of lymphotrophic herpes viruses. *Immunol Today* **17:** 198.

Mendez E, Kawanashi T, Clemens K, Siomo H, Calabresi PA, Brady J, Jacobson J (1997). Astrocyte Specific Expression of Human T-Cell Lymphotrophic Type I (HTLV-1) Tax: Induction of TNF alpha and Susceptibility to Lysis by CD8+ HTLV-1 Specific Cytotoxic T Cells. *J Virology* **71:** 9143–9149.

Merrill JE, Benveniste EN (1996). Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* **19:** 331–338.

Merrill JE, Murphy SP (1997). Inflammatory events at the blood brain barrier: regulation of adhesion molecules, cytokines, and chemokines by reactive nitrogen and oxygen species. *Brain Behav Immunol* **11:** 245–263.

Nakagawa M, Izumo S, Ijichi S, Kubota H, Arimura K, Kawabata M, Osame M (1995). HTLV-1 associated myelopathy: analysis of 213 patients based on clinical features and laboratory findings. *J Neurovirol* **1:** 50–61.

Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR (1998). The Chemokine Receptors CXCR3 and CCR5 Mark Subsets of T Cells Associated with Certain Inflammatory Reactions. *J Clin Invest* **101:** 746–754.

Raine CS (1994). Multiple Sclerosis: Immune system molecule expression in the central nervous system. *J Neuropathol Exp Neurol* **53:** 328–337.

Ransohoff RM, Glabinski A, Tani M (1996). Chemokines in immune-mediated inflammation of the central nervous system. *Cytokine & Growth Factor Rev* **7:** 35–46.

Rieckmann P, Albrecht M, Kitze B, Weber T, Tumani H, Broocks A, Luer W, Helwig A, Poser S (1995). Tumor necrosis factor-α mRNA expression in patients with relapsing-remitting multiple sclerosis is associated with disease activity. *Ann Neurol* **37:** 82–88.

Sallusto F, Lenig D, Mckay CR, Lanzavecchia A (1998). Flexible Programs of Chemokine Receptor Expression on Human Polarized T Helper 1 and 2 Lymphocytes. *J Exp Med* **187:** 875–883.

Schall T (1994). The Chemokines. *The Cytokine Handbook.* London, Academic Press: 419–460.

Simpson JE, Newcombe J, Cuzner ML, Woodroofe MN (1998). Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. *J Neuroimmunol* **84:** 238–249.

Smith GL (1996). Virus proteins that bind cytokines, chemokines or interferons. *Curr Opin Immunol* **8:** 467–471.

Soldan S, Bert R, Salem N, Secchiero P, Calabresi PA, Maloni H, McFarland HF, Lin HC, Patnaik M, Jacobson S (1997). Association of human herpes virus 6 (HHV-6) with multiple sclerosis: Increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nature Med* **3:** 1394–1397.

Stuve O, Chabot S, Jung SS, Williams G, Wee Yong V (1997). Chemokine-enhanced migration of human peripheral blood mononuclear cells is antagonized by interferon beta-1b through an effect on matrix metalloproteinase-9. *J Neuroimmunol* **80:** 38–46.

Stuve O, Dooley NP, Uhm JH, Antel JP, Francis GS, Williams G, Wee Yong V (1996). Interferon β-1b decreases the migration of T lymphocytes in vitro: Effects on matrix metalloproteinase-9. *Ann Neurol* **40:** 853–863.

Tanaka Y, Mine S, Figdor C, Wake A, Hirano H, Tsudada J, Aso M, Fujii K, Saito K, Van Kooyk Y, Eto S (1998). Constitutive chemokine production results in activation of leukocyte function-associated antigen-1 on adult T-cell leukemia cells. *Blood* **91:** 3909–3919.

Taub D (1996). Chemokine-Leukocyte Interactions. *Cytokine and Growth Factor Rev* **7:** 355–376.

Umehara F, Izumo S, Takeya M, Takahashi K, Sato E, Osame M (1996). Expression of adhesion molecules and monocyte chemoattractant protein-1 (MCP-1) in the spinal cord lesions in HTLV-I-associated myelopathy. *Acta Neuropathol (Berl)* **91:** 343–350.

Zou Y, Kottmann A, Kuroda M, Taniuchi I, Littman D (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* **393:** 595–599.