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Cytokines and Chemokines in the Pathogenesis of Experimental Autoimmune Encephalomyelitis

William J. Karpus

Experimental autoimmune encephalomyelitis is a CD4+ T cell–mediated demyelinating disease of the CNS that serves as a model for multiple sclerosis. Cytokines and chemokines shape Th1 and Th17 effector responses as well as regulate migration of leukocytes to the CNS during disease. The CNS cellular infiltrate consists of Ag-specific and nonspecific CD4+ and CD8+ T cells, neutrophils, B cells, monocytes, macrophages, and dendritic cells. The mechanism of immune-mediated inflammation in experimental autoimmune encephalomyelitis has been extensively studied in an effort to develop therapeutic modalities for multiple sclerosis and, indeed, has provided insight in modern drug discovery. The present Brief Review highlights critical pathogenic aspects of cytokines and chemokines involved in generation of effector T cell responses and migration of inflammatory cells to the CNS. Select cytokines and chemokines are certainly important in the regulatory response, which involves T regulatory, B regulatory, and myeloid-derived suppressor cells. However, that discussion is beyond the scope of this brief review. The Journal of Immunology, 2020, 204: 316–326.

In contrast to multiple sclerosis (MS), which arises spontaneously in patients, experimental autoimmune encephalomyelitis (EAE) is most commonly induced by active immunization with peptides derived from myelin proteins emulsified in CFA or adoptive transfer of myelin-specific T cells. Therefore, the induction of EAE requires Ag processing, presentation, and cytokine-mediated steps in secondary lymphoid tissue prior to the generation of a fully encephalitogenic T cell capable of migrating to the CNS and initiating the pathogenic cascade. The most commonly used encephalitogenic peptides for inducing EAE are MOG35-55 for mice on the C57BL/6 background and PLP135-151 for the SJL mouse strain, and they are physically transported in an adjuvant-containing emulsion from the s.c. injection site to the draining lymph nodes (LN) by dendritic cells (DC) (1–5). The migration of these cells to the LN requires a leukocyte trafficking event where CCR6 is downregulated and CCR7 is upregulated (6, 7). LN express CCL19 and CCL21, both of which are ligands for CCR7 and mediate lymphocyte and DC homing to LN (8, 9). Although CCR2 has been demonstrated to be important for the generation of Th1 responses and migration of DC to the LN in Leishmania infection (10, 11), it is not clear whether this is required for APC migration to LN in EAE induction. It was once thought that chemokines were deposited on glycosaminoglycan molecules and served as a pathway that guided migrating leukocytes; however, recent research suggests that this may not be the case, and leukocytes most likely sense increasing concentrations of soluble chemokines (12).

Upon uptake of peptide Ag and interaction of their pathogen-associated molecular patterns with TLR agonists such as peptidoglycan, which is found in CFA, APC acquire an activated phenotype that results in altered expression of chemokines and chemokine receptors that functions to enhance the local inflammatory response prior to their migration to LN (13). Once in the LN, the peptide-loaded APC presents Ag to specific MHC class II–restricted T cells and in the presence of the appropriate costimulatory signal, sets the program of Th1/Th17 differentiation in motion. Costimulation required a functional CD28–CD80/86 interaction. If blocked, the result can be induction of immune tolerance and amelioration of EAE (14, 15). A number of cytokines produced by APC are necessary to drive the differentiation program toward the pathogenic phenotype. EAE was long considered a Th1-driven disease in which APC production of IL-12 (16) induced T-bet–expressing Th1 cells (17, 18) that secreted IFN-γ (19), which subsequently activated macrophages as end-stage effector cells in demyelinating disease (20). Surprisingly, mice deficient for either IFN-γ (21) or IFN-γR (22) developed more-severe CNS inflammatory disease. This led to a shift in the investigation of pathogenic T cell development and effector function. Later studies focused on dissecting the APC molecular contribution to T effector cell differentiation and indicated that in the absence of IL-12 p35, mice were susceptible to EAE, whereas IL-12 p40–deficient mice were resistant to EAE (23, 24). These results suggested an alternative APC mechanism for the induction of an encephalitogenic T cell phenotype. These observations...
led to the discovery that IL-23 shared the p40 subunit with IL-12 (25) and that APC expression of either IL-23 p19 or p40 was critical to the differentiation of Th17 cells and development of EAE (26–29). Following upregulation of the transcription factors RORyt and RORα, induced by IL-23, IL-6, and TGF-β (30, 31), T cells that express IFN-γ and IL-17 have the ability to adoptively transfer severe EAE (29). Additional investigation suggested that following TCR-induced proliferation, TGF-β and IL-6 provided a stimulus that conferred a Th17 phenotype (32–34). A number of additional studies suggest IL-1RII signaling through IL-1RI (35) as well as IL-21 (36, 37) also play a role in Th17 differentiation, and thus development of EAE, presumably through regulation of IL-23R expression (31). The functional significance of generating an IL-17 response with respect to EAE development may lie in the suggestion that this effector cytokine induces IL-6, IL-1β, CXCL2, and CCL2 production, which in turns leads to an inflammatory cascade resulting in attraction of both T cells and myeloid-derived leukocytes to the CNS (38).

Despite the numerous studies demonstrating the association of Th17 effector cells with development and progression of EAE, observations were made that subsets of these cells do not induce disease (39, 40). This led to the demonstration that some Th17 cells produce IL-10, whereas others produced GM-CSF, TNF-α, and IL-22, despite the latter not being required for EAE development (41). GM-CSF, however, appears to play a critical role in disease as mice deficient for GM-CSF are resistant to EAE development (42). Moreover, EAE could be adoptively induced by transfer of IL-17A– or IFN-γ–deficient T cells but not with GM-CSF–deficient T cells (40), providing evidence of the requirement of the latter as a critical effector cytokine. Indeed, expression of GM-CSF results in the migration of CCR2-expressing monocytes into the CNS (43) as well as in the activation and maturation of microglia, which are critical to EAE development and progression (44). TNF-α synergizes with both IL-17 and GM-CSF to enhance chemokine expression by a variety of cell types (45), and mice deficient for TNF-α demonstrated decreased EAE with concomitant reductions in expression of critical chemokines and chemokine receptors (46). Once pathogenic T cells are activated and acquire their effector functionality in LN, they upregulate sphingosine-1-phosphate receptor (SIP1R), which allows them to leave the LN and enter the blood stream (47, 48). In addition to regulating T cell egress from LN, SIP1R signaling in APC can regulate the balance between Th1 and T regulatory cell (Treg) populations (49), alter the trafficking patterns of Th1 and Treg (50), and affect Th17 differentiation by modulating IL-6 expression (51).

Chemokines and chemokine receptors in CNS leukocyte infiltration

How T cells as well as other leukocyte populations, such as monocytes, neutrophils, and DC, enter the CNS during disease pathogenesis has been intensely investigated over the past three decades. According to the three-step model of leukocyte migration across tissue barriers (52), chemokines [reviewed in Ref. (53)] have been postulated to activate integrins (54) as well as induce the directional migration of specific chemokine receptor–expressing leukocytes (55). A number of these ligands and receptors [reviewed in Ref. (56)] are associated with EAE (57–60), although only a subset has been determined to be necessary for development of EAE, with spatial and temporal expression patterns being a major factor in functional inflammatory outcome (61).

Initial entry of T cells into the CNS requires an interaction between CD29/CD49d (αvβ3 integrins) expression on the T cells (62) with CD106 (VCAM-1) expressed on cerebrovascular endothelial cells. Treatment of mice with anti-αvβ3 mAb at the initiation of disease has been shown to prevent development of CNS inflammation and subsequent clinical disease (63). There is evidence that CD29/CD49d and one of the CXCL12 receptors, CXCR7, may be cooperatively involved in early CNS T cell entry (64), presumably by a mechanism other than enhancement of integrin avidity. The importance of other T cell–expressed integrins, such as CD11a/CD18 (αvβ2 integrins) (65, 66), in the disease process was similarly demonstrated using mAb therapy. Whether CD11a/CD18 is required for the migration of T cells into the CNS to initiate disease or whether it serves as an adhesion molecule during Ag presentation and subsequent T cell reactivation in the CNS has not been conclusively determined. A role for selectins in the initial T cell migration into the CNS has not been demonstrated, although CD62L may function during later steps of disease pathogenesis (67), perhaps by stabilizing the location effector cells in the CNS during the demyelinating process (68). The concept of CNS leukocyte infiltration occurring as a regulated, stepwise process beginning with Ag-specific T cell infiltration and proceeding with infiltration of monocytes, macrophages, and other myeloid-derived cells (such as neutrophils and DC) as well as Ag-nonspecific T cells and T cells specific for spread epitopes culminating in the formation of tertiary lymphoid structures in situ forms the basis of the current understanding of EAE pathogenesis. The initial accumulation of Th1 and Th17 cells in the CNS is the prelude to severe inflammation and self-tissue damage with subsequent clinical manifestation (Fig. 1).

Chemokines have been postulated to be required for leukocyte integrin activation (69) and subsequent transendothelial migration (70). However, in the absence of local inflammation, most chemokines are not expressed in the CNS (59). The exceptions to this are CXCL11, which is constitutively expressed by neurons and endothelium in the brain (71), and CXCL12, which is both constitutively expressed as well as induced by a variety of cell types in the CNS (72). CXCL12 interaction with one of its receptors, CXCR4, has been shown to regulate the spatial pattern of inflammatory infiltrate. An intact CXCL12–CXCR4 axis resulted in the typical perivascular inflammatory cell accumulation pattern, whereas inhibition of CXCR4 signaling resulted in a more-diffuse parenchymal infiltrate (73). Alternatively, it is conceivable that initial T cell entry to the CNS does not require a chemokine signal. One alternative explanation is that neuroantigen-specific CD4+ T cells enter the CNS in the absence of a chemokine signal from local tissue and induce the recruitment of additional Ag-specific and Ag-nonspecific T cells as well as macrophages by secreting or inducing CNS cells to secrete chemokines. Some early evidence supported the idea that PLP139–151–specific CD4+ T cell clones expressing the chemokines CCL1 and CCL5 in response to neuroantigen-specific stimulation were able to elicit clinical disease following adoptive transfer, whereas those clones that...
did not express chemokines did not induce EAE (74). The second possibility is that encephalitogenic CD4+ T cells, through expression of cytokines such as IFN-γ, IL-17, GM-CSF, TNF-α, and/or lymphotoxin, induce either the cerebrovascular endothelium, astrocytes, or microglia to secrete chemokines, which in turn regulates T cell migration into

FIGURE 1. Summary of cytokines and chemokines critical to EAE pathogenesis. (A) Upon Ag uptake and activation, APC downregulate CCR6 and upregulate CCR7 (1) to respond to CCL19 and CCL21 expressed in LN and traffic to T cell–rich areas. APC present encephalitogenic peptides to T cells and secrete IL-6, IL-23, and TGF-β (2) that drive Th17 differentiation. Th17 cells downregulate CCR7 (3) to leave LN and enter the bloodstream. Th17 cells express CCR2 and CCR6 and are capable of secreting the effector cytokines IL-17 and GM-CSF as well as TNF (4). (B) Effector leukocytes, including encephalitogenic T cells, monocytes, and neutrophils, travel in the bloodstream (5) and roll on cerebrovascular endothelium as well as interact with endothelium-bound CCL2 via expression of CCR2. Effector leukocytes extravasate through the endothelium (6) and enter the CNS perivascular and parenchymal spaces. Activated Th17 cells and monocytes secrete cytokines, such as IL-17, GM-CSF, and TNF, that activate astrocytes (7) and resident microglia and perivascular macrophages to produce chemokines, such as CCL2, CCL3, CCL19, CCL20, CCL21, CCL22, CXCL1, CXCL2, and CXCL10, that amplify the migration and accumulation of leukocytes (8). Effector leukocytes produce cytokines and enzymes (9) as well as make physical contact with axons, resulting in demyelination and subsequent clinical disease symptoms.
CNS. Indeed, the signaling cascades resulting from IFN-\(\gamma\), IL-17, and TNF can transcriptionally activate or potentiate chemokine expression (75–83). Several candidate chemokines, such as CXCL1, CCL2, and CCL5, have been shown to be expressed in a variety of cerebrovascular endothelial model systems (84–86). In these examples, endothelial chemokine expression was augmented by proinflammatory cytokine stimulation, thereby suggesting that encephalitogenic Th1/Th17 cells producing IFN-\(\gamma\), IL-17, GM-CSF, and/or TNF-\(\alpha\) have the ability to stimulate chemokine expression by cerebrovascular endothelium. However, murine EAE does not require IFN-\(\gamma\) expression, as mice deficient for this gene product are able to develop the disease (21), indicating that multiple inflammatory factors may have the capacity to induce endothelial chemokine expression. CCL2 expression by cerebrovascular microvessels as well as perivascular astrocytes has been demonstrated in vivo, thus making it plausible that one of the first events in encephalitogenic T cell migration into the CNS occurs at the blood brain barrier (84, 87, 88).

The evidence that chemokines were associated with the development of EAE derived from a number of studies demonstrating an association between mRNA expression and clinical disease development. In a rat model of acute EAE in which animals develop a single disease episode followed by spontaneous remission, it was shown that CCL2 mRNA was temporally expressed in the CNS of rats with disease and that when the animals entered remission, CCL2 mRNA could no longer be detected (89). In experiments in which both the actively and adoptively induced mouse models of EAE were employed, it was demonstrated that CNS CCL1, CCL2, CCL3, CCL4, CCL5, CXCL10, and CXCL12 mRNA expression remained elevated throughout the course of acute clinical disease signs (90). Using an MOG peptide-induced model of acute EAE in the C57BL/6 mouse, it has also been shown that CCL2 and CCL5 expression correlated with clinical disease severity (91).

Because there is a large body of evidence suggesting that chemokines and their receptors function to regulate encephalitogenic T cell migration during EAE, the pathological process of how encephalitogenic cells get into the CNS has been the focus of many studies. The biological significance of chemokine expression in the pathogenesis of EAE was initially demonstrated using in vivo chemokine-specific Ab treatment approaches (92–95) and subsequently by specific genetic approaches. CNS CCL3 and CXCL10, but not CCL2, protein expression was shown to correlate with increasing EAE severity, and anti-CCL3 (92) as well as anti-CXCL10 (96), but not anti-CCL2 or anti-CCL5, treatment prevented acute clinical EAE in SJL mice. Furthermore, in vivo anti-CCL3 or anti-CXCL10 treatment reduced accumulation of encephalitogenic T cells, host-derived bystander T cells, and monocytes in the CNS (92, 97). These studies demonstrated that CCL3 and CXCL10 are important factors in the pathogenesis of acute EAE in SJL mice and suggested an ability to directly induce CNS T cell accumulation. However, acute EAE appears to be controlled by a different chemokine expression program in C57BL/6 mice. The fact that CCL2 is associated with acute EAE development in this particular model has been shown by ligand expression, ligand neutralization, and genetic ablation studies (98, 99). CCL2 can be produced by CNS astrocytes and microglia as well as by infiltrating T cells and macrophages, with the CNS-derived, as opposed to the infiltrating cell–derived, source of the chemokine being important for disease progression (100, 101). Moreover, studies inducing EAE in CCR2-deficient mice as well as specific adoptive transfer of CCR2-deficient T cells strongly inferred a role for the ligand CCL2 in the disease process (102–105). These studies indicate that CCL2 signaling appears to regulate the migration of T cells (105) in EAE development; however, there is a suggestion that T cell migration can occur via CCL2 through a CCR2-independent pathway (106). Neither CCL3 (107) nor CCL5 (95) appears to play a role in a C57BL/6 model of EAE, and mice deficient for CCR3, which is a receptor for CCL3 and CCL5, also showed no decrease in EAE development (107). Yet, mice deficient for CCR1, which is also a receptor for CCL3 and CCL5, showed a partial decrease in the severity of acute EAE (108), presumably through a mechanism involving macrophage, rather than T cell, migration. CXCL10 (96), but not CXCL9 (95), was shown to be important in the development of acute EAE in SJL mice using an Ab-mediated neutralization approach. Similarly, CXCR3, the receptor for CXCL9, 10, and 11 expressed by activated T cells, was also shown to be important for disease development using a receptor blockade approach (109). In contrast, neither the CXCL10- (110) nor the CXCR3-deficient (111, 112) mouse on a C57BL/6 background showed a reduction in clinical disease; rather, they both showed either similar or exacerbated disease. The lack of CXCR3 expression on astrocytes may explain the enhanced disease seen in global CXCR3-deficient mice due to an inability to dampen the Th17 response in the CNS (112, 113). Th17 cells in EAE have been shown to express CCR6, which has been postulated to functionally regulate migration to CNS and subsequent disease development (114, 115), although expression is not limited to this functional cell type, as B cells (116), Tregs (117), innate lymphoid cells (118), and regulatory DC (119) also express the receptor. The ligand for CCR6, CCL20, is expressed in the CNS during EAE (120). However, given the wide cellular distribution of CCR6 expression, conclusions about its specific role in EAE pathogenesis should be made with caution, as all cell-specific outcomes have not been fully parsed.

Because the majority of MS patients present as relapsing-remitting, the clinically relevant question in EAE arises as to what chemokines are produced in the CNS during the relapses and which of those chemokines drive disease relapses. The CNS of (SJL × SWR)F1 mice with chronic relapsing EAE was examined for the expression of chemokines as a function of time after disease induction (58). There was a dramatic increase in CCL2 mRNA and protein expression in the brain and an increase in CCL2 mRNA expression in the spinal cord during the relapsing phase of disease. CCL3 mRNA expression in the spinal cord remained elevated from the acute EAE episode through the relapsing phase of disease. Furthermore, CCL2 expression was localized to astrocytes, whereas CCL3 expression was localized to the perivascular mononuclear cell infiltrate. The fact that CCL2 plays a biologically relevant role in the relapsing EAE disease process was demonstrated in SJL mice by a differential expression pattern whereby acute disease showed no correlation, whereas relapsing disease correlated with expression. Acute disease in SJL mice was inhibited by treatments with anti-CCL3, whereas relapsing clinical EAE was ameliorated with anti-CCL2 treatment,
with both treatments resulting in reduced accumulation of leukocytes in the CNS (94). It is also possible that acute and relapsing disease may be differentially regulated as a result of alternative signaling outcomes for chemokines other than cell migration, including modulation of T cell differentiation (121–126). To that end, when CCL2 was overexpressed in the CNS, the result was decreased EAE with an associated reduction in Th1 responses (127). Therefore, it is possible that differential chemokine expression during different phases of disease has multiple outcomes that affect disease pathology.

Leukocyte populations other than T cells infiltrate the CNS during EAE (128), and chemokines and their receptors also appear to play an important role in the migration and accumulation of these subpopulations. CCR2-deficient mice immunized with MOG peptide and monitored for the development of disease did not develop clinical EAE, showed no CNS mononuclear cell infiltration, and had indistinguishable T cell cytokine responses from control mice; rather, development of disease did not develop clinical EAE, showed a lack of CNS monocyte accumulation (98). The results of these two studies indicate a very critical role for CNS CCL2 expression and corresponding CCR2 expression on monocytes/macrophages in the development of EAE. It is well accepted that macrophages express CCR2, and the major chemotactic function is induced by the ligand CCL2 (129, 130). Not surprisingly, macrophages have been divided into subpopulations based on their function (131). Inflammatory, or M1, macrophages presumably are major pathogenic mediators of EAE and, in addition to CCR2, they express CCR4, whereas anti-inflammatory macrophages do not express CCR4 (132, 133). Genetic ablation of CCL17 (134) or immunoneutralization of CCL22 (135), both of which are ligands for CCR4, resulted in attenuation of EAE (136). Similarly, mice deficient for CCR4 did not develop severe EAE (137, 138). The majority of the evidence in these studies indicated a role for CCR4 in the migration and functionality of inflammatory macrophages. Both CCL19 and CCL21 are ligands for CCR7, have been shown to be upregulated in the CNS during the development of EAE (139, 140), and have been suggested to be important in the development of encephalitogenic Th17 cells (141). Given these results, it was somewhat surprising that CCR7 deletion did not result in alteration of EAE (142). However, a more-recent report using a T cell–specific CCR7 deletion approach demonstrated a role for CCR7 in the priming of those encephalitogenic T cells rather than an alteration in migration to the CNS (143).

A number of studies have suggested that following entry into the CNS, pathogenic T cells have to be reactivated in situ by recognizing myelin Ag peptide in the context of MHC class II (144) plus costimulation to become fully encephalitogenic (145) by infiltrating or resident DC (146). Indeed, CCR2 has been shown to be critical for the migration of DC to the CNS to regulate in situ encephalitogenic T cell reactivation (147). Additional evidence suggests that CNS infiltration of encephalitogenic Th17 cells results in parenchymal expression of CXCL1 and CXCL2, both of which are ligands for CXCR2 (148, 149). Furthermore, CXCR2 is expressed by neutrophils (150) and inflammatory monocytes (57, 151), and CXCR2-deficient mice fail to develop EAE (148). The same study demonstrated that transfer of wild-type neutrophils into CXCR2-deficient mice can restore disease development, confirming a critical role for neutrophils that express CXCR2 in EAE pathogenesis that was postulated a number of years ago (152). Numerous investigations have gone on to suggest that spatial CXCR2 signaling is critical to the CNS localization of inflammatory cells and subsequent subsets of clinicopathological outcomes (153–155). The role of NK cells in both trafficking to the CNS and in the effector mechanisms of EAE is not well understood; however, they appear to require CX3CR1 expression, as mice functionally deficient for this molecule show markedly reduced NK cell numbers in the CNS and no changes T cells, NKT cells, and monocyte/macrophages (156, 157).

Much of the early experimental work describing the entry of encephalitogenic T cells into the CNS focused on the perivascular infiltration in the parenchyma. However, CNS-infiltrating leukocytes, including T cells, have been demonstrated at meningeal locations (158–160). This location-specific process is regulated by innate lymphoid cells (161) and mast cells (162, 163) that express cytokines and chemokines related to disease pathogenesis. One of the key features of meningeal infiltration is the establishment of tertiary lymphoid organs in which the production of CXCL13 serves to attract CXCR5-expressing B cells (164). In addition to B cells (165), T follicular helper cells also express CXCR5, respond to CXCL13, and populate the tertiary lymphoid structures (166). CXCL13 has been shown to be expressed in the spinal cords of mice with EAE, and genetic ablation of this chemokine resulted in less-severe disease (167, 168). The function of B cells in EAE appears to be more important in the relapsing-remitting model in which presentation of myelin-derived Ag by B cells in the process of epitope spreading has been postulated to drive the relapsing episodes of clinical disease (169).

Targeting cytokines and chemokines for disease therapy

Much of the investigation into the role of cytokines and chemokines, as well as their receptors, in EAE has revealed a number of pathologic mechanisms that have arguably shaped our understanding of the disease process in MS and how to develop new therapeutic approaches (170, 171). The fact that select cytokines and chemokines have been shown to be critical to the development of EAE presents an opportunity to target these molecules for therapeutic intervention and potential advancement to clinical trials for assessment of efficacy in the treatment of MS. Of all the aforementioned cytokines and chemokines/chemokine receptors associated with EAE pathology, a limited subset emerges as probable therapeutic targets with translatability to MS treatment. The importance of IL-17 to the induction of EAE was shown by the ability of anti–IL-17 to abrogate disease (172, 173). The effects of anti–IL-17 on EAE in a monkey model were much less pronounced (174). Importantly, anti–IL-17 (secukinumab) has been approved for use in psorias (175) and is still being studied in MS clinical trials (176). Similarly, anti–IL-23 treatment inhibits EAE (177), but targeting IL-12/23 p40 in MS patients with ustekinumab was less effective than available therapies (178) and, therefore, not likely to be further considered. Anti–GM-CSF treatment has also been shown to reduce severity of EAE (179), and a small phase
The importance of the CCL2–CCR2 axis in both acute and relapsing EAE, presumably by regulating the migration of T cells, monocytes, macrophages, and DC, underscored the possibility of targeting chemokine receptors for inhibition of disease development and progression. Three specific approaches have been used to target chemokine ligand–receptor pairs: development of a small m.w. receptor antagonist, construction of a modified chemokine ligand that binds and blocks the receptor without inducing downstream signaling, and production of neutralizing Abs to either the ligand or the receptor. Small-molecule inhibitors for CCR1 (180, 181) were developed that had limited efficacy in preventing the development of EAE (182) but showed no efficacy for MS therapy (183), presumably because of the requirement for high and constant receptor occupancy (184). Small-molecule antagonists were developed for CCR2 that bound the receptor, displaced specific chemokine binding, and inhibited downstream signaling as well as mouse EAE (185, 186). Similarly, a CXCR7 antagonist also prevented development of EAE (187), whereas antagonism of CXCR4 led to increased CNS infiltrate (73), demonstrating differential outcomes for receptors that can both bind CXCL12. Small-molecule antagonism of CXCR3 (188) as well as CCR4 (189, 190) led to reduced EAE. A CCR7-specific small molecule has also been identified and used for the inhibition of EAE. In this particular study, rather than inhibiting the migration of T cells to the CNS, the postulated mechanism of action was upregulation of CCR7 cell surface expression that subsequently allowed pathogenic T cells to alternatively migrate to LN (191).

The modified chemokine approach for inhibition of chemokine receptor signaling and therapy for EAE has been used with some efficacy. Amino terminal modification of CCL5 by addition of a methionine (met-RANTES) showed a modest effect of decreasing EAE (192). This inhibitor is able to antagonize CCR1 and CCR5, and because CCR5 does not appear to play a role in EAE (107), the mechanism of action was presumed to be inhibition of CCR1-mediated inflammatory cell migration. McColl and colleagues (109) have also used the approach of modified chemokine proteins as inhibitors of specific chemokine receptor function and demonstrated that inhibiting CXCR3 and CXCR4 or CCR6 resulted in reduced EAE (193).

The third therapeutic approach for targeting chemokine receptors in EAE by developing mAbs has also been employed. Anti-CXCR3 (194) treatment showed some effects in disease inhibition as did anti-CCR6 (193). The results of these studies were interpreted as inhibition of encephalitogenic T cell trafficking to the CNS or inhibition of DC migration to lymphoid tissue and interruption in the Ag presentation process. However, the possibility that inhibition of chemokine receptor signaling affected the ability to generate an encephalitogenic phenotype cannot be ruled out (123, 195). Another interesting approach has been used to target and neutralize chemokines for the inhibition and treatment of EAE. Vaccination of mice with DNA vectors encoding specific chemokines, such as CCL2 and CCL3 (196) as well as CXCL10 (197), resulted in protection from severe EAE development. This experimental intervention relies on the host’s ability to mount a neutralizing immune response against its own chemokines. Interestingly, vectors encoding CCL4 or CCL5 did not protect from development of EAE.

Conclusions

The present brief review summarizes the role of cytokines and chemokines in the pathogenesis of EAE. It should be pointed out that there are many soluble and cell-associated molecules that have been shown to be associated with development and/or progression of EAE that were not covered in this report. That by no means minimizes their importance; rather, it reinforces the idea of immense complexity in the immunological interactions for both pathogenic and regulatory processes of autoimmune disease. Although the pathologic process of EAE appears to involve many different molecules expressed by many cell types, a small subset has emerged as critical to disease development and, by extension, initiated intense drug discovery efforts for translation into viable therapies for MS. Some of these candidates include IL-6, IL-23, IL-1β, and TGF-β that play significant roles in driving self-antigen specific T cells to acquire an encephalitogenic phenotype. Once activated, the encephalitogenic T cells produced cytokines and chemokines, such as IL-17, GM-CSF, and CCL2, that are critical in their ability to further induce inflammation that includes induction of additional chemokines that attract myeloid-derived leukocytes. CCR2, CCR6, and CXCR2 are chemokine receptors that have emerged as major players in the inflammatory cascade. By extension, their ligands are also important in inflammation. Although chemokine receptor expression may be fairly stable on leukocyte populations, chemokines are more dynamically regulated. We previously postulated that spatial and temporal chemokine regulation was the key to induction of inflammation.

When considering cytokines and chemokines, as well as chemokine receptors, there are three major challenges going forward that must be overcome to successfully translate findings in EAE to therapies for MS. The first challenge is that all of the cytokine and chemokines covered in this review that are major factors in EAE pathogenesis have also evolved to be critical elements of the host defense response against pathogens. Therefore, by interfering with their function, one also creates a situation in which host defense is compromised. This challenge will require significantly more research into the spatial and temporal cytokine- and chemokine-mediated mechanisms of autoimmune pathogenesis so that precise, rather than global, therapeutic approaches can be developed. The second major challenge is that in both the cytokine and chemokine systems, evolution has resulted in redundant systems, such that there are few examples in which inactivation of one ligand or receptor incapacitates the host response. This is particularly evident in the chemokine system in which a particular leukocyte subpopulation may express a diverse array of chemokine receptors, receptors may bind more than one ligand, and ligands may bind different receptors. However, a number of examples in the present review illustrate that there is lack of redundancy, and these have been exploited for therapeutic benefit in EAE. The third challenge relates to the fact that not all pathogenic mechanisms involved in EAE are mirrored in the pathogenesis of MS. This is further complicated.
by the use of inbred mouse models compared with genetically divergent humans. Moreover, the experimental methodologies used to study cytokines and chemokines in the pathogenesis of EAE often involve a reductionist, rather than a systems, approach. Nevertheless, much has been learned about human MS through the study of cytokines and chemokines in EAE, and a wealth of new information of how the critical molecules interact to produce complex disease phenotypes is waiting to be revealed.

**Disclosures**

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