Chemokines and disease

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We examine here several diseases that are associated with inappropriate activation of the chemokine network. Detailed comment has been restricted to pathological states for which there are compelling data either from clinical observations or animal models. These include cardiovascular disease, allergic inflammatory disease, transplantation, neuroinflammation, cancer and HIV-associated disease. Discussion focuses on therapeutic directions in which the rapidly evolving chemokine field appears to be headed.

Comparative phylogenetic analyses suggest the presence of a rudimentary chemokine system in *Drosophila* and primitive vertebrates. This ancient system has since evolved coincidentally with immune effector cells. In their earliest avatar, chemokines were probably mediators of innate immune-cell trafficking and directors of cell movement during morphogenesis. As more advanced vertebrate phyla emerged, gene duplication established a rich repertoire of chemokines and receptors. Selective pressures imposed on host defense by unique pathogen–commensal microbe relationships created a robust system of overlapping ligands and receptors that protect the host but, as in most host defense systems, maladaptive responses may result in injury.

The known chemokine system in humans comprises approximately 50 ligands (http://cytokine.medic.kumamoto-u.ac.jp/) and 20 G protein–coupled receptors. The principal targets of chemokines are bone marrow–derived cells. Because motility is an essential part of their function, chemokines play a central role in leukocyte physiology by controlling basal and inflammatory trafficking. However, the functional consequences of chemokine receptor activation are not limited to locomotion. Granule exocytosis, gene transcription, mitogenic effects and apoptosis are also affected by chemokines (see the review by Thelen[1] in this issue of *Nature Immunology*). In addition, many other cell types also express chemokine receptors. These include endothelia, smooth muscle cells, stromal cells, neurons and epithelial cells[2]. Thus, in addition to localizing cells of the immune system to particular compartments, chemokines may be involved in other aspects of tissue homeostasis.

Chemokine expression in disease

Chemokines can be divided broadly into two categories: inducible chemokines that recruit leukocytes in response to physiological stress and constitutive chemokines that are responsible for basal leukocyte trafficking and forming the architecture of secondary lymphoid organs. Although simplistic and inaccurate in its details, this generalization nonetheless provides insight into how members of this family cause disease. Expression of inducible chemokines can be elicited by almost any stimulus that alters cellular homeostasis and mRNA encoding induced chemokines can increase over 300-fold within a few hours of activation. Thus inducible chemokines can be thought of as a vertebrate cellular "SOS response" that recruits leukocytes to areas of tissue injury.

However, their "hair-trigger" inducibility and high expression also create the potential for persistent or inappropriately "exuberant" expression. In addition, collateral damage wrought by activated leukocytes may not dampen the chemokine stimulus that initiated their recruitment. In fact, leukocyte-mediated injury may simply induce higher expression of these or even new chemokines, creating a feed-forward scenario that results in more extensive tissue damage. In this way, the protective effects of leukocytes can be subverted in a manner that causes disease. This is the context in which chemokine inhibition or antagonism makes therapeutic sense. However, it should be remembered that in some diseases— for example, HIV—chemokine agonists might also be beneficial.

The primary motivation for examining chemokines in disease is the ease with which their expression can be documented in disorders associated with leukocyte infiltration (**Table 1**). However, as attractive as it may be to hypothesize that chemokine expression is responsible for pathology, the only human disease for which there is incontrovertible evidence for an association with the chemokines system is HIV infection. In all other cases, definitive roles for chemokines in human pathogenesis or pathobiology have been inferred from animal models. So, in this review, we will restrict our discussion to diseases in which animal models show a role for chemokines and for which correlative data exist in humans.

Chemokines and receptors: duets *versus* symphonies

The question of chemokine redundancy must be addressed early in any discussion of their potential role in disease. The striking target-cell specificity of chemokines persuaded most early investigators of a "one chemokine–one cell type" paradigm. But, as more chemokines and receptors were discovered, it became clear that there was enormous overlap in ligand-receptor specificity, which led many in the field to suggest that the chemokine system was rife with redundancy. However, gene-targeting approaches have provided new insights into the biology of chemokines. In some cases, such as eosinophilic pneumonia, one ligand–receptor pair may perform a self-contained "duet" that produces a pathologically straightforward disease[3]. However, in most cases, temporal and spatial patterns of chemokine and receptor expression are interwoven with ligand-receptor specificity to result in a multicomponent pathophysiological response. The resulting "symphony" of interactions leads to the cooperative activity of several cell types. Understanding the role that each chemokine plays in this orchestra will be essential for determining which ones will be the best therapeutic targets in a particular disease.

Multiple sclerosis

Multiple sclerosis (MS) is a chronic relapsing neuroinflammatory disease in which inappropriate recognition of an autoantigen on myelinated

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Table 1. Chemokines and disease

Category	Human diseases	Animal models
Autoimmune disease	Rheumatoid arthritis; systemic lupus erythematosis; MS	Autoimmune arthritis (for example, collagen-induced arthritis); MRL-*Fas*^{lpr}; experimental allergic encephalitis
Graft rejection	Heart allograft rejection; kidney allograft rejection	Heterotopic heart allografts; sponge allografts
Infection	Acute and chronic bacterial and viral infections (especially HIV and mycobacteria); sepsis	Rodent models using the same or analogous pathogens; cecal ligation and puncture
Inflammation or allergy	Asthma; arthritis; colitis; psoriasis	Antigen sensitization and anatomically specific delivery (for example, inhaled antigen challenge in asthma models)
Neoplasia	Leukocyte recruitment in cancer; angiogenesis	Therapeutic vaccination; *in vivo* angiogenesis models
Vascular	Atherosclerosis; hypertension; ischemia-reperfusion	Hypercholesterolemic rodents; genetic models of hypertension; ischemia-reperfusion

Rodent models in which chemokines have been shown to play a role can be divided into five general categories as indicated. The human diseases relevant to the rodent models within each category are shown.

nerve fibers recruits T lymphocytes and macrophages into the central nervous system. In an active MS lesion, perivascular mononuclear cells localize near an area of active inflammation that is characterized by reactive astrocytes and glial cells. The disease has periods of remission, which implies the existence of regulatory immune mechanisms. Currently, corticosteroids are the main treatment for active disease but, consistent with its immune basis, treatment with interferon β (IFN-β) reduces the frequency of relapses.

Chemokines in EAE

The best animal model for MS is experimental autoimmune encephalomyelitis (EAE)[4]. It can be induced by immunization using antigens derived from myelin, such as proteolipid protein (PLP) fragments and myelin oligodendrocyte glycoprotein (MOG). When appropriately administered, these antigens elicit an acute demyelinating process driven by T cells, which can have a chronic relapsing course that is similar to MS. Several reports indicate that the appearance of regulated upon activation, normal T cell–expressed and secreted (RANTES), macrophage-inflammatory protein 1α (MIP-1α), MIP-1β, IFN-γ–inducible protein 10 (IP-10) and monocyte-chemoattractant protein 1 (MCP-1) mRNA and protein correlate with inflammatory lesions[5–10]. A particularly interesting early study that used neutralizing antibodies in the chronic relapsing PLP model suggested that MIP-1α was associated with acute onset of inflammatory lesions, whereas MCP-1 was associated with relapse[6].

Based on these results, mice that are deficient in MIP-1α, MCP-1, CC chemokine receptor 1 (CCR1), CCR2 or CCR5 have been used to investigate EAE. Both MIP-1α–deficient and CCR5-deficient mice contracted MOG-induced disease that was indistinguishable from that contracted by wild-type mice[11], although CCR1-deficient mice had a decreased incidence and less severe clinical score[12]. In contrast, two groups have shown that CCR2-deficient mice are almost completely protected from MOG-induced disease, despite some discrepancies between the two sets of data[13,14]. It has also been shown that peripheral lymphocytes from CCR2$^{-/-}$ mice responded to secondary MOG challenge with normal IFN-γ secretion and that adoptive transfer of T cells from sensitized CCR2$^{-/-}$ mice induced disease. In contrast to this, however, sensitized T cells from CCR2$^{-/-}$ mice did not produce IFN-γ in response to restimulation, which was consistent with a description of generalized T_H1 deficiency in CCR2$^{-/-}$ mice[15]. Explanations for these contradictory results have not yet been given.

Finally, mice deficient in MCP-1 are also resistant to MOG-induced EAE. T cells from sensitized MCP-1$^{-/-}$ mice produce disease when transferred to wild-type mice but cells from sensitized wild-type mice cannot produce disease when transferred to MCP-1$^{-/-}$ mice. As in CCR2-deficient mice, T cells from sensitized MCP-1$^{-/-}$ mice secrete less IFN-γ than cells from wild-type mice. Thus MCP-1 appears to be the important ligand for CCR2 in this disease model. Although these results may appear to contradict recent reports of the importance of MCP-1 in mounting T helper 2 (T_H2) responses[16], the explanation lies in the nature of the disease model. PLP and MOG immunization produces overwhelmingly T_H1-polarized responses so that the role of MCP-1 in T_H cell polarization may not be relevant. Instead, MCP-1 is likely to be critically important in eliciting CCR2-expressing effector cells, such as macrophages, to produce manifestations of disease. Thus, animal modeling has pointed to MCP-1 and CCR2 and, to a lesser extent, MIP-1α and CCR1 as potential therapeutic targets in the treatment of MS.

Chemokines in human MS

A large amount of observational data indicates that MS in humans may be influenced by many of the same chemokines that have been highlighted in rodent systems. For example, increased amounts of MIP-1α have been found in the cerebrospinal fluid (CSF) of relapsing patients, although similar elevations were found in other neuroinflammatory disorders as well[17]. MCP-1, MCP-2 and MCP-3 have been found in active MS lesions from autopsied brains with the greatest immunochemical concentration occurring at the center of the lesion. In addition, it has been noted that MCP-1 and MCP-3 decorate lesional vessels that have perivascular infiltrates[18].

Consistent with these findings is the presence of cognate receptors CCR2 and CCR5 on foamy macrophages, activated microglia and T cells in lesions[19]. CCR3 has also been described as being present on these cells as well as on reactive astrocytes, which also express CCR5 but not CCR2. In addition, there are increased amounts of IP-10, MIG and RANTES in the CSF of patients suffering active attacks of MS. IP-10 and MIG are also present in the active lesions within autopsied brains, both in plaque-associated macrophages and reactive astrocytes. Notably, the receptor for IP-10, CXCR3, has been described as being present on virtually all perivascular T cells and astrocytes associated with active lesions[20]. Finally, it has also been found that T cells in the CSF of patients with active disease were highly enriched for those that express CXCR3 or CCR5 compared to peripheral T cells[21].

These observations suggest that the expression of chemokines such as IP-10, MIG or RANTES by lesion-associated cells may be involved in the recruitment of specific T cells expressing CXCR3 or CCR5. A hint that at least part of this chemokine axis may be important in human disease comes from the observation that patients treated with IFN-β

had lower serum RANTES concentrations[22]. The well described "experiment of nature" whereby approximately 20% of northern Europeans carry an inactive CCR5 allele (Δ32) permits questions to be asked about the role of this receptor in human MS. In an Australian study of 120 MS patients and age-matched controls, two individuals with MS were found to be homozygous for the Δ32 allele. Clearly, then, CCR5 is not necessary for the development of MS[23]. However, in families affected by MS, the Δ32 allele was associated with an approximate 3-year delay in onset of disease[24].

Transplantation

Because the chemokine system plays an essential role in host defense, it is not surprising that chemokines and their receptors may be involved in rejection of allogeneic transplants[25]. Chemokines could influence at least three aspects of allograft biology[26]. First, restoration of blood flow in the allograft could lead to ischemia-reperfusion injury in which chemokines recruit leukocytes. Second, host responses to infection during immune suppression involve chemokines. Third, the inflammatory components of acute and chronic rejection are likely to be controlled by chemokines.

Animal allograft models

In heart and skin transplants, an early mixed inflammatory response that is characterized by neutrophil and monocyte infiltration is observed. Concurrently, neutrophil-active chemokines, such as MIP-2 and KC, and monocyte-active chemokines, such as MCP-1, appear. This occurs both in syngeneic and allogeneic transplants and is a response to ischemia-reperfusion. However, several days after allogeneic transplant, a new pattern of chemokine expression is observed. While MCP-1 expression persists, IP-10, MIG and intereron-inducible T cell α chemoattractant (I-TAC), which are all ligands for CXCR3, appear along with MIP-1β and RANTES, which are ligands for CCR5[27–32]. This assortment of chemokines may be necessary for orchestrating the movement of cells involved in acute rejection. These cells include $CD4^+$ and $CD8^+$ T lymphocytes, macrophages, natural killer (NK) cells and antigen-presenting cells (APCs), which traffic between the graft and regional lymph nodes.

Analysis of mice carrying targeted deletions of these chemokines and their receptors has started to clarify their relative importance in allograft rejection (**Fig. 1**). For example, heterotopic cardiac transplants across major histocompatibility complex (MHC) class I and class II barriers survived twice as long in $CCR1^{-/-}$ mice compared to wild-type mice (14 *versus* 7 days). Remarkably, low doses of cyclosporin A, which would ordinarily be insufficient to prolong graft survival, produced permanent engraftment (survival beyond 200 days) in $CCR1^{-/-}$ recipients. In addition, chronic rejection and graft arteriosclerosis were not observed in $CCR1^{-/-}$ mice treated with cyclosporin A or anti-CD4[33].

These analyses have been extended to mice that are deficient in CCR2, CCR5, CXCR4 and IP-10[25]. $CCR2^{-/-}$ recipients of cardiac allografts had a phenotype similar to that of $CCR1^{-/-}$ mice. Also, their graft survival time was doubled in the absence of additional immunosuppression. In the same setting, $CCR5^{-/-}$ recipients showed a tripling of graft survival time. However, the most impressive response was observed in mice that were deficient in CXCR3. In the absence of additional intervention, allograft survival extended to 55–60 days. Again, supplemental treatment with pulsed or low doses of cyclosporin A resulted in permanent engraftment without evidence for chronic rejection or graft arteriosclerosis[34].

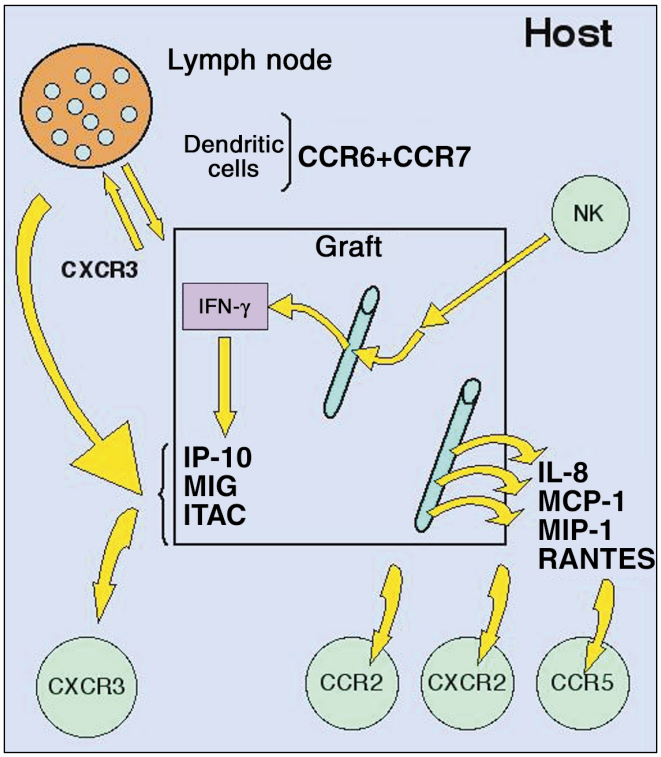

Figure 1. Chemokines involved in allograft rejection. Early nonspecific release of several chemokines attracts CCR2-, CXCR2- and CCR5-bearing leukocytes. Wandering recipient NK cells survey MHC mismatches at the vascular endothelium and respond by producing IFN-γ. Local synthesis of the CXCR3 ligands IP-10, MIG and I-TAC occurs, recruiting $CXCR3^+$ T cells and plasmacytoid dendritic cells. Recipient cells invade the graft, which results in acute and chronic rejection. Thereafter, graft arteriosclerosis, probably driven by CXCR3 and MCP-1, progress independently.

Of the CXCR3 ligands, IP-10 appears 3 days after transplant, before the onset of acute rejection. This may be in response to IFN-γ secretion by NK cells when they encounter the graft. By day 7, when acute rejection is maximal, IP-10 decreases but MIG and I-TAC appear. Although each of these ligands activates CXCR3, their sequential appearance implies that they have distinct functions that, for now, are unclear. Nonetheless, compared to allografts in wild-type hosts, those in $CXCR3^{-/-}$ mice at day 7 have a substantially reduced number of $CD4^+$, $CD8^+$, $CD25^+$ and $CD45^+$ cells and decreased macrophage infiltration. Plasmacytoid dendritic cells also express CXCR3[35] and the absence of this receptor may affect the trafficking of APCs during rejection.

Another aspect of allograft rejection that may be influenced by chemokines is graft arteriosclerosis. It is distinct from lipid-driven atherosclerosis and is characterized by concentric intimal smooth muscle cell proliferation and uniform perivascular inflammatory cell infiltration that is rich in T cells and macrophages. Induction of tolerance and graft acceptance by anti-CD40–CD40L or disruption of CD40 does not abolish graft artheriosclerosis[36]. Smooth muscle hyperplasia may be directly dependent on local IFN-γ because this process occurs in the absence of recipient leukocytes[37]. Hypothetically, this IFN-γ could come from CXCR3-expressing activated donor T or NK cells or macrophages, the recruitment of which would depend on local chemokine expression. CXCR3 antagonism may be as attractive in this setting as it is in allograft rejection even though the pathophysiology of these processes differs.

HIV

Until 1995, chemokines were the exclusive property of a subset of immunologists and pathologists who were interested in inflammation. Today, the number of Medline entries under "chemokine" exceeds 11,000 and 90% were published within the last 5 years. The most visible of these new citations have been in the field of HIV pathophysiology. As reviewed elsewhere, a flurry of discoveries led to the identification of CXCR4 and CCR5 as obligate coreceptors with CD4 in viral envelope fusion and entry[38]. This involves a sequential two-step process in which the interaction of gp120 with CD4 opens a cryptic binding site on the latter for the chemokine receptor. Binding energy, which is provided by coreceptor interaction, contributes to the conformational rearrangement that exposes the fusion peptide gp41[39,40].

The proof-of-principle for the role of CCR5 in HIV pathogenesis lies in demonstration of the fact that the majority of exposed-uninfected individuals are deficient in cell surface CCR5 expression owing to homozygous carriage of the Δ32 deletion. Many different human populations have a surprisingly high prevalence of this and other nonfunctional CCR5 alleles[41,42]. But what selective advantage might accrue to a CCR5-deficient individual or population? Small studies in humans have suggested that absence of CCR5 provides no overt protection from viruses other than HIV. Instead, there is evidence that Δ32 is associated with a reduced risk of asthma, a less severe clinical course of rheumatoid arthritis and, as noted above, a later onset of MS. It should be noted, however, that, as in MS, an absence of CCR5 does not prevent the appearance of rheumatoid arthritis. In contrast to these protective effects of CCR5 loss, there is a report that Δ32 allele frequencies are elevated in patients suffering from a clinically severe form of sarcoidosis compared to patients with more benign disease[43]. Alternatively, selective pressure by an epidemic infectious disease, such as bubonic plague, might account for the high allele frequency of Δ32 among Europeans if CCR5 were required for full manifestation of disease. These considerations will influence decisions about the clinical acceptability of CCR5 antagonists.

The risk-benefit calculation for a CCR5 antagonist in HIV is complicated but may be summarized as follows. First, there is sparse evidence that absence of CCR5 either promotes disease or is associated with increased severity of disease. Second, there is genetic proof that the CCR5-deficient state is protective in HIV disease. Although this calculus results in an overwhelmingly favorable argument for the safety and efficacy of CCR5 antagonism, some caveats should be heeded. At least three reports describe infection of homozygous CCR5-deficient patients by CXCR4-utilizing viruses, which indicates that primary infection can occur in the absence of CCR5. In addition, it has been reported that the frequency of CXCR4-utilizing viral isolates increases in individuals who are heterozygous for Δ32[44]. Thus there is a concern that CCR5 antagonism may predispose patients to changes in viral tropism with the attendant increased pathogenicity associated with T tropic viruses. However, it should be noted that such a shift in tropism would require ongoing viral replication that may be prevented by highly active antiretroviral therapy (HAART).

Several pharmaceutical companies have been engaged in a search for CCR5 antagonists with an eye toward indications in HIV disease. A few teams have succeeded in making small-molecule CCR5 antagonists that block HIV-1 entry *in vivo*. One molecule, which has an extremely attractive toxicity profile, is in Phase I trials[45]. Thus, it is likely that the first real anti-chemokine drug to be used in humans will target HIV disease, although associated therapies would need to include post-exposure prophylaxis because such a drug would essentially render an individual CCR5-negative. Despite this, such anti-chemokine therapy would provide a desirable adjunct to HAART. In addition, a CCR5 antagonist might permit lower doses of HAART, thereby reducing toxicity. Finally, it might be used to facilitate a "drug holiday" from HAART, which, it has been suggested, is an effective strategy in boosting a cytotoxic T lymphocyte response against the virus.

Asthma

Asthma is a chronic inflammatory disease of small airways that is characterized by mononuclear, eosinophil and mast cell infiltration of the submucosa along with mucous gland hyperplasia and subepithelial fibrosis. Disease exacerbations arise from stimuli that are allergic, such as exposure to cockroach antigen, and nonallergic, such as viral infections. Although the asthma pathogenesis paradigm favors a role for T_H2 cells and eosinophils, other cells, such as mast cells and basophils, are also involved. A key laboratory finding concerning asthma is airway hyperresponsiveness (that is, smooth muscle contraction) to nonspecific stimuli. Its biochemical mechanism is unclear but may be related to the presence of T lymphocytes and eosinophils and the cytokines they secrete, such as interleukin 4 (IL-4), IL-5, IL-9 and IL-13. Because chemokines influence inflammatory-cell trafficking and contribute to shaping the immune response, much effort has been devoted toward documenting a role for chemokines in human asthmatic patients[46–48].

Chemokines in rodent airway hypersensitivity models

Many investigators have studied airway responses in rats, mice and guinea pigs that have been sensitized to ovalbumin and then challenged with antigen delivered by aerosol. In mice, this protocol leads to mononuclear cell and eosinophil infiltration of the lung and airway hyperresponsiveness. However, these rodent models are not particularly faithful to human asthma. They should more properly be considered as models for hypersensitivity pneumonitis and/or alveolitis, which differ from human disease because they have essentially no alveolar component. In addition, the degree of airway hyperresponsiveness in the mouse model underestimates that which is observed in humans. Thus, the mouse model is best used to understand the roles of particular cytokines and chemokines in cell trafficking. In a detailed study that involved multiple chemokines and receptors, serial waves of chemokine and receptor interactions that began with the sensitization phase and continued through the challenge phase were shown[49]. Through the use of neutralizing antisera, it was shown that MCP-5, eotaxin, RANTES and MCP-1 contribute in a nonredundant fashion to airway responsiveness and cellular emigration. A pan–β chemokine antagonist protein from the poxvirus family was also shown to block inflammation in this model[50], as was Met-RANTES[51] (a CCR1 and CCR3 antagonist).

Surprisingly, however, $CCR1^{-/-}$, $CCR4^{-/-}$ and eotaxin-deficient mice are not protected in this model (A. Humbles and C. Gerard, unpublished data). And, even though disruption of CCR3 reduces airway eosinophil accumulation by 50%, $CCR3^{-/-}$ mice actually show enhanced bronchial constriction in response to methacholine, one of the hallmarks of asthmatic hyperreactivity. Thus, the data generated by antibody neutralization are not entirely consistent with observations of knockout mice. Possible explanations include differences among the aerosol protocols and the kinetics of cell recruitment. Rather than studying physiology after a single challenge, studies have been performed on consecutive days to maximize inflammation. These serial antigen aerosol challenges have been associated with a progression of dependence on CCR3 to CCR4[52]. Thus, in the contrived model, the importance of each of these receptors may be masked.

Nonetheless, targeting the T lymphocyte chemokine receptors in asthma may be a reasonable strategy because adoptive transfer of sensitized T cells can produce pulmonary hypersensitivity in recipient mice. In addition, as noted earlier, a small study suggests that the Δ32 allele of CCR5 may be associated with protection from developing asthma. T_H2-polarized cells have been shown to express CCR4 and CCR8, which suggests that they may provide targets for anti-asthma therapy[53,54].

Rheumatoid arthritis

This debilitating disease is characterized by the presence of a mixed inflammatory cell infiltrate into synovium-lined joints in response to an, as yet unidentified, autoantigen. A critical role for tumor necrosis factor (TNF) is indicated by the success of anti-TNFs and soluble receptor antagonists but a significant clinical need remains for orally active small-molecule inhibitors. TNF induces the expression of many chemokines and targeting these downstream mediators of TNF activity is a rational therapeutic goal.

Rodent models of arthritis

There are relatively few proof-of-principle studies in mouse arthritis models. As with asthma, murine models for arthritis are pale surrogates for human disease and include adjuvant-induced, septic, collagen-immune complex, pristane and MRL-*Fas*^{lpr} arthritides, among others. MCP-1 and CCR2 are implicated in joint destruction because antagonism of MCP-1 reduced the prevalence and severity of autoimmune adjuvant–induced arthritis in MRL-*Fas*^{lpr} mice[55]. Neutralization of MCP-1 modified disease in rats[56] and met-RANTES was effective, presumably because it blocked CCR1 and CCR5[57]. Because this is a T_H1-modulated disease, CCR5 and CXCR3 are reasonable targets, although CCR1, CXCR4 and CXCR1 are also candidates. These hypotheses will almost certainly be tested in the relevant knockout models.

Chemokines in human arthritis

Synovial fluid from actively involved joints contains a number of chemokines, including MCP-1, MIP-1α, IL-8, RANTES and IP-10. Both synovial-lining cells and infiltrating leukocytes are the source of these chemokines. CCR2, CCR5, CXCR2 and CXCR3 have been documented as appearing on infiltrating cells[58–65]. Again, one large study indicated that the Δ32 CCR5 allele was found less frequently in patients with rheumatoid arthritis[66]. A second study found Δ32 at the expected frequency among patients with rheumatoid arthritis, although its presence was associated with a milder clinical course and was more frequently associated with absence of rheumatoid factor[67]. These observations suggest that targeting CCR5 might prove beneficial.

Neoplasia

Despite a long history of investigation, the role of leukocyte infiltration in cancer remains uncertain. The report that first described the presence of leukocytes in human tumors inferred that cancers arose from these and other connective tissue cells at sites of inflammation[68]. However, it rapidly became apparent that tumors themselves elicit leukocytes by secreting chemotactic factors, in particular MCP-1[69–74].

There are at least two models for the function of tumor-associated leukocytes, both of which have experimental support. On one hand, they are a potential source of growth factors for tumor cells and angiogenic factors for endothelial cells. In murine models, mixing macrophages with syngeneic tumor cells results in tumor formation by extremely low inocula that would not otherwise "take"[75]. In human tissues, a correlation has been noted between the extent of macrophage infiltration of breast cancers and their degree of vascularity, which suggests that leukocytes may contribute to tumor angiogenesis and, hence, tumor survival[76,77]. In these studies, macrophage infiltration was an independent predictor for early relapse. In contrast, MCP-1 expression also matches the degree of macrophage infiltration in human cervical cancers but both of these parameters decrease as the grade of cervical intraepithelial neoplasia advances[77].

On the other hand, tumor-associated leukocytes may be residual evidence of the host's ineffective attempt to reject the tumor immunologically. If this is the case, enhancing this response might lead to tumor eradication. Several chemokines have been used in animal models to elicit tumor-specific immune responses that result in tumor rejection. These include MCP-1[79–81], RANTES[82], TCA-3[83], MIP-3α[84], SLC[85], IP-10[86] and lymphotactin[87]. Whether chemokines have any greater efficacy than granulocyte-macrophage–colony stimulating factor (GM-CSF) in this system will have to await the results of trials in humans.

The potential relevance of chemokines to malignancy extends beyond their roles in leukocyte recruitment. Murine models show that chemokine secretion by tumor cells themselves may influence angiogenesis and, secondarily, tumor growth. CXC chemokines that contain the three-amino-acid glutamate-leucine-arginine motif have angiogenic activity in corneal micropocket assays, whereas CXC chemokines without this motif are antiangiogenic[88]. Expression of angiogenic CXC chemokines by tumor cells in severe-combined immunodeficient (SCID)–human chimeras or in syngeneic mice enhances tumor growth, whereas the expression of antiangiogenic chemokines is inhibitory[89]. A correlation between the expression of angiogenic chemokines and non-small-cell lung cancer growth has been observed in humans[90].

There are also reports that some chemokines may act directly as growth factors for tumor cells. For example melanoma growth stimulatory activity (MGSA) stimulates the proliferation of melanoma and pancreatic cell lines[91,92] and IL-8 has been reported to do the same for non-small-cell lung cancer cells[93]. However, the relevance of these findings to human neoplasia must be viewed with some caution. The cardinal insight into oncogenesis during the past 15 years is that it is a genetic disease and thus an extensive database of genetic and chromosomal abnormalities in human and murine cancers has been developed. However, so far, none of these abnormalities maps to chemokine or chemokine receptor loci.

One more potential role for chemokines in cancer may be related to metastatic behavior. Several malignant cell types express chemokine receptors[94–96]. Although these receptors transduce signals, their true function remains unknown. As discussed earlier, chemokines and their receptors are likely to be involved in morphogenetic movements during organogenesis. It is possible that malignant transformation may result in the reappearance of some receptors that had been involved in this process earlier in ontogeny. If tumor cells invade the circulation, one unfortunate consequence of chemokine receptor expression might be the arrest and invasion of these cells in response to chemokine ligand expression by cells in another organ. The result would be metastatic invasion. Although there are as yet no data that directly support this model, it would be another example of a pathological outcome arising from subversion of the chemokine system.

Vascular disease

One of the most exciting areas of chemokine biology is cardiovascular disease. In particular, accumulating evidence indicates that these mediators may play a central role in atherosclerosis. A general model has emerged in which atherosclerotic plaques are thought to result from an inflammatory response to arterial damage that occurs

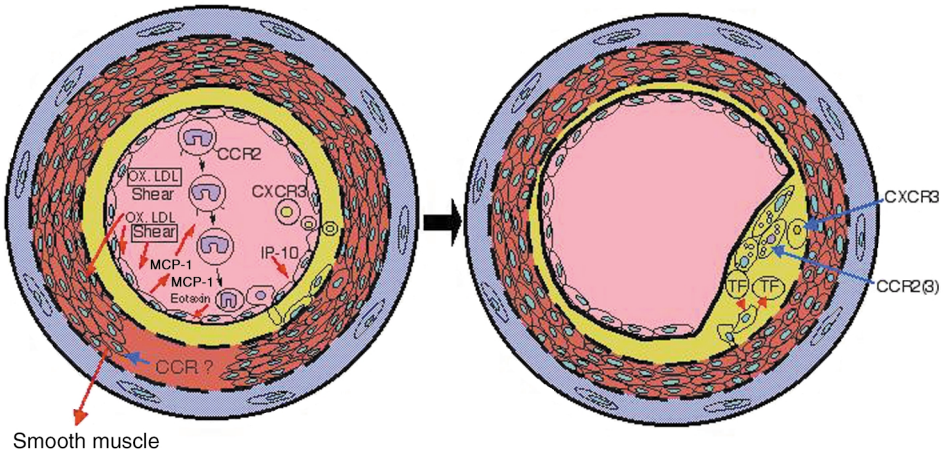

Figure 2. Chemokines and atherosclerosis. Oxidized lipid (OX-LDL) or flow shear stress (Shear) from hypertension or disordered blood flow produces endothelial cell damage and induces secretion of chemokines, in particular MCP-1. MCP-1 can also be secreted by smooth muscle cells in response to physical stretch or to growth factors such as platelet-derived growth factor (PDGF) released from platelets (not shown). Circulating monocytes engaged in selectin-mediated rolling along the endothelium encounter MCP-1 and are stimulated to engage in firm adhesion followed by diapedesis into the subendothelium. Here, monocytes differentiate into macrophages, which take up lipid and become the foam cells within the fatty streak. In addition, IP-10 synthesis attracts CXCR3$^+$ T lymphocytes into the lesion. Also shown is MCP-1–inducible expression of tissue factor (TF) by smooth muscle cells, which contributes to the prothrombotic nature of the plaque. Because these cells do not express CCR2, it is suggested that an unknown receptor (CCR?) mediates this response as well as, perhaps, the migration of these cells into the intima.

because of hypercholesterolemia or the shear stresses of hypertension or disordered blood flow[97] (**Fig. 2**). In a paradigmatic example of the multistep process of leukocyte emigration, activated endothelial or arterial smooth muscle cells are thought to release chemokines that induce firm adhesion of monocytes rolling along the vascular endothelium. This is followed by their diapedesis into the subendothelium where, as macrophages, they take up lipid and become the foam cells within the fatty streak. Chemokines may also be involved in smooth muscle migration into the intima and, ultimately, in thrombus formation over the plaque. This picture of atherosclerosis as an inflammatory disease has great explanatory power and permits the mechanistic inclusion of infectious agents thought to be involved in atherogenesis[98]. These include cytomegalovirus (CMV), which encodes an MCP-1– and RANTES-responsive chemokine receptor called US28 (which is expressed by infected smooth muscle cells)[99] and *Chlamydia pneumoniae*, which activates MCP-1 expression when it infects endothelial cells[100].

IL-8, stromal-derived factor 1 (SDF-1), IP–10, I309 and CXCR2 have all been associated with lesions in animal models of atherosclerosis[101–104]. However, the most compelling pathogenetic case has been made for the ligand-receptor pair of MCP-1 and CCR2. MCP-1$^{-/-}$ mice have 65–85% less arterial lipid deposition than MCP-1$^{+/+}$ mice in hypercholesterolemia models such as low density lipoprotein (LDL) receptor deficiency or apoB overexpression[105,106]. CCR2$^{-/-}$ mice show a similar reduction in disease within an apoE-deficiency model[107,108]. In all cases, reduction in disease was accompanied by a concomitant reduction in the macrophage content of the arterial wall. This suggests that the role of MCP-1 in vascular disease is to attract CCR2-bearing monocytes into the vessel wall where continued lipid ingestion leads to plaque formation.

Consistent with that model is the observation that CCR2 is required for macrophage infiltration into the intima in experimentally induced hypertension in mice[109]. However, it should be noted that apoE-deficient mice develop LDL-reactive T cells that become progressively T_H2 polarized as disease advances[110]. Considering the role of MCP-1 in stimulating T_H2 responses[15], it is possible that MCP-1–deficient mice may have less atherosclerotic disease because they are unable to mount a T_H2-polarized anti-LDL response. However, this mechanism would not apply to CCR2-deficient mice because they are fully capable of T_H2 responses.

The relevance of these observations to human atherosclerosis is primarily suggested by demonstrations of chemokine expression in human lesions. For example, the presence of MCP-1 within human atherosclerotic plaques has been documented[111,112]. In addition, RANTES, LD78 and eotaxin are present in plaques, although their functional roles are more difficult to infer because of the absence of data from animal models[113,114].

Other aspects of atherosclerosis pathophysiology may also be controlled by chemokines. It has been shown that extremely low concentrations of MCP-1 induce the expression of tissue factor by human arterial smooth muscle cells[115]. This suggests that MCP-1 may contribute not only to the accumulation of monocytes and macrophages in the plaque but also to the plaque's thrombotic character, which plays such an important role in acute coronary syndromes. Notably, these cells do not express detectable CCR2 mRNA, which suggests that MCP-1 may recognize another receptor on these cells.

Although there are currently no therapeutic interventions directed specifically at chemokines in vascular disease, it is worth noting that members of the statin family of HMGCoA reductase inhibitors also inhibit expression of MCP-1[116,117]. Thus, in addition to their lipid lowering effects, statins may also reduce atherosclerotic risk by down-modulating MCP-1–mediated macrophage recruitment in the arterial wall. Finally, an interesting proof-of-principle for therapeutic targeting of MCP-1 in atherosclerosis has recently been reported in an animal model. An engineered MCP-1 mutant, called 7ND, has been described in which the removal of amino acids 2–8 created a potent MCP-1 antagonist. Intramuscular delivery of a 7ND expression plasmid in rats resulted in sustained plasma 7ND and prevention of monocyte recruitment in a coronary artery remodeling system[118].

Conclusions

At first glance, the suggestion that components of host defense are involved in disease might seem internally inconsistent. However, this paradigm has been established in other systems and is well suited to chemokines because of their direct effects on activating and recruiting leukocytes. One aspect of chemokine physiology that makes these proteins and their receptors especially attractive therapeutic targets is their specificity. Unlike cytokines, which have pleiotropic effects, chemokines target specific leukocyte subsets and, in some settings, may only attract these cells without activating them. Antagonism of a

single chemokine ligand or receptor would be expected to have a relatively circumscribed effect, thereby endowing the antagonist with a limited side effect profile. For example, corticosteroids are highly effective and toxic anti-inflammatory agents that exert their effects, at least in part, by suppressing chemokine expression. A specific chemokine antagonist with clinical efficacy would have a much higher therapeutic index than steroids. The challenge now is to develop small-molecule antagonists with good bioavailability, that is, drugs. When available, these agents will undoubtedly expand the universe of chemokine-based diseases beyond the very limited selection we have discussed here.

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