Chemokines are a family of structurally related low molecular weight (8–10 kDa) proteins that are important for the organization of tissues during development and regulate cell motility and localization both during development and in the adult. In the adult, this function is predominately related to the trafficking of leukocytes, although more recently the impact of these molecules on other cell types has become apparent. Chemokines mediate their effects by binding seven transmembrane, G-protein coupled, receptors. In addition to their primary role in regulating cell motility, they can also influence cell survival and proliferation. Antagonists for a number of chemokine receptor have been developed, raising the possibility of interfering with chemokine function as a therapeutic tool. This review focuses on the emerging roles for chemokines in normal physiology and disease.

**Key words:** Chemokines, Chemokine receptors, Immune system, Malignancy, Hematopoiesis

Chemokines and their receptors

Chemokines share a common basic structure consisting of three anti-parallel β-strands and an overlying α-helix. However, chemokines can be divided into 4 structural classes defined by the spacing of the first two conserved cysteine residues of these proteins (Table 1). The first family described, which includes CXCL8, was the alpha or CXC family where these cysteine residues are separated by a single amino acid. In the largest group, the beta or CC family, the first 2 cysteines are immediately adjacent. The remaining classes are smaller with the gamma and delta families having only 2 and 1 member/s respectively. In the gamma or C chemokines, lymphotactin (or XCL1) and SCM1β (XCL2), only one of the 2 conserved cysteines has been retained resulting in a single disulphide bond. Lastly the sole CX3C chemokine, fractakine/neurotactin has 3 amino acids separating the 2 cysteines. Chemokines can also be classified according to their primary function. Most chemokines are primarily involved in inflammatory responses (inflammatory chemokines), others are principally involved in homeostatic functions (homeostatic chemokines) while others perform functions related to both processed (dual function chemokines). In addition to acting as agonists, amino terminal truncation of many chemokines results in their inactivation and in some instances, including CCL2 (9-67) and CCL7 (5-76), results in antagonistic activity (McQuibban et al., 2000, 2002), suggesting that proteolytic cleavage of chemokines may act to terminate an inflammatory response.

Chemokine receptors are members of a class of seven-transmembrane G protein-coupled receptors proteins and form part of a much larger superfamily including receptors for hormones, neurotransmitters, paracrine substances and inflammatory mediators. There have been at least 18 chemokine receptors identified to date, but with nearly 50 ligands, receptor promiscuity is obvious. However infidelity is restricted to within families with six receptors binding CXC chemokines, 10 CC, and one each to CX3C and C chemokines. Not only do multiple chemokines share a single receptor but frequently individual chemokines bind multiple receptors. Despite the general receptor promiscuity a few monogamous chemokine/ligand interactions have been identified including CXCL12/CXCR4 and CX3CL1/CX3CR1. Polymorphisms of receptors has been observed with the most well described being the inactivating CCR5Δ32 mutation (Samson et al., 1996). Chemokine-receptor binding induces signalling mediated through coupled heterodimeric G proteins, resulting in a wide variety of downstream biological activities, including chemotaxis, alterations in calcium flux, induction of leucocyte degranulation and phagocytosis, and respiratory burst (Johnson et al., 2004).

Chemokines and their receptors in human physiology

**Normal hematopoiesis**

Studies from knockout (KO) mice have
demonstrated that most chemokine receptors are not essential for life, with all but one of these animals being at least superficially normal. The exceptions is the CXCL12 KO mouse, which die perinatally, with defects in numerous organ systems (Nagasawa et al., 1996; Ma et al., 1998; Tachibana et al., 1998; Zou et al., 1998). CXCL12 has a single receptor CXCR4 and the CXCR4 knockout animals share an identical phenotype to the CXCL12 knockout animals with malformations of the intestinal vasculature, cardiac ventricular septal defects and abnormal migration of cerebellar neurons (Ma et al., 1998; Tachibana et al., 1998; Zou et al., 1998). Examination of the hematopoietic compartment in these animals reveals severely reduced numbers of B and myeloid cell progenitors in the bone marrow and reduced numbers of B cell progenitors in the fetal liver.

Table 1. Chemokines and their receptors.

<table>
<thead>
<tr>
<th>LIGAND</th>
<th>ALTERNATE NAMES</th>
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<tbody>
<tr>
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<td></td>
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<tr>
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<td>CX3CL1</td>
<td>I</td>
<td>CX3CR1</td>
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</table>

I: inflammatory, H: homeostatic, D: Dual function.
Expression of CXCR4 by hematopoietic progenitor cells and the production of CXCL12 by bone marrow stromal cells and osteoblasts (Lapidot, 2001; Netelenbos et al., 2003) is important for the homing of hematopoietic progenitor cells (HPC) to the bone marrow, both during ontogeny and in the adult, and for the retention of these cells within this organ (Kawabata et al., 1999; Lapidot, 2001). CXCR4 function is also required for normal maturation of myeloid and lymphoid cells (Ma et al., 1998, 1999; Zou et al., 1998; Kawabata et al., 1999; McGrath et al., 1999; Onai et al., 2000; Egawa et al., 2001; Glodek et al., 2003) with CXCL12 synergising with other cytokines such as stem cell factor (SCF) and interleukin (IL)-7 to enhance the survival and proliferation of B cell precursors and myeloid progenitors cells and independently enhancing the survival of myeloid progenitors (Lee et al., 2002; Broxmeyer et al., 2003). The phenotype of the CCR9 KO mouse suggests that this receptor also plays a significant, although not essential role, in the development of B cells in the bone marrow (Wurbel et al., 2001).

The regulation of the development of T cells is less clear and a generalized summary is shown in Figure 1. Although CXCR4 is required for in vitro survival, expansion and differentiation of early human thymocytes, and synergises with IL-7 to enhance the expansion of these cells (Hernandez-Lopez et al., 2002), in vivo models have been less convincing. Adoptive transfer of CXCR4 KO fetal liver cells into wild-type recipients has not consistently demonstrated T cell defects (Kawabata et al., 1999; Ma et al., 1999), although CXCL12 intrakine mice displayed a partial arrest of thymocyte maturation from the double negative to the double positive state (Onai et al., 2000). CCR9 also appears to play a significant, although not essential role, in T cell development in the bone marrow (Wurbel et al., 2001). No single chemokine predominates T cell maturation in the thymus, with the sequential expression of several chemokine receptors regulating the movement of immature T cells within the thymus and various microenvironmental niches being associated with distinct chemokine expression patterns (Nagasawa, 2000; Annunziato et al., 2001; Savino et al., 2002). However, CCR7 is required for the proper development of secondary lymph nodes (Forster et al., 1999) and CXCR5 for that of the germinal centres and Peyers patches (Forster et al., 1996). Newly created naïve T cells express CCR7, which facilitates their homing to peripheral lymph nodes but does not provide access to non-lymphoid peripheral tissues. CCL21 and CCL19 are expressed on the luminal side of high endothelial venules (HEV) in lymph nodes and facilitates the entry of naive T cells into these tissues (Williams and Butcher, 1997). Contact with dendritic cells (DCs) within the lymph node induces CXCR5 expression on CD4+ T cells and drives these cells to the margins of follicular zones where they provide follicular B cell help (Breitfeld et al., 2000; Schaeerli et al., 2000). Effector cells are produced during an adaptive immune response, and peripheral effector T cells and effector memory cells largely lack expression of CCR7, a property which is reacquired by central memory cells and is probably related to their return to lymph nodes (Sallusto et al., 1999). Th1 cells predominately express CCR5, CXCR3 and CXCR6, and while Th2 cells preferentially express CXCR3 and, less specifically, following activation CXCR4 and CCR8 (Sallusto et al., 1998). The role of these receptors in the function of Th cells is at best poorly understood, with chemokine promiscuity between these receptors further diminishing any suggestion of specific targeted functions.

Cells of the innate immune system, including phagocytes, monocytes, macrophages and natural killer cells, undergo a simpler migratory program during their development within the bone marrow and their later participation in immune responses in the periphery. These cells are armed with chemokine receptors, including CXCR1 and 2, and CCR1, 2, 3, 5 and 10 that permit their early recruitment to sites of infection (Ley, 2003).

Immune cell trafficking

The mechanisms underlying immune surveillance in the periphery, by antigen presenting cells and memory T cells, is also poorly understood. CXCR4 activates integrin function and thereby facilitates the tight adhesion of rolling T cells on activated endothelial cells and their subsequent extravasation (Campbell et al., 1998b; Ding et al., 2000; Grabovsky et al., 2000; Kantele et al., 2000). The ability of CXCL12 to guide the movements of mature B cells, plasma cells, (Burger et al., 1999; Casamayor-Palleja et al., 2001; Hargreaves et al., 2000) and CD4+ T cells (Williams and Butcher, 1997) to secondary lymph nodes. The expression of CXCR5 on CD8+ T cells, which facilitates their homing to the T cell zones in secondary lymph nodes, is required for the efficient control of viral infections (Sallusto et al., 1998). CXCR4 is also expressed on the surface of activated CD8+ T cells and serves as a co-receptor for the chemokine CXCL12, which is important for the retention of these cells in the bone marrow (Lapidot et al., 1999). CXCR4 is also expressed on the surface of activated CD8+ T cells and serves as a co-receptor for the chemokine CXCL12, which is important for the retention of these cells in the bone marrow (Lapidot et al., 1999).
et al., 2001; Luther et al., 2002; Nakayama et al., 2003), T cells (Sawada et al., 1998) and dendritic cells (Zou et al., 2001; Luther et al., 2002) in vitro suggests that CXCR4 may act as a non-redundant cell-signaling system essential to immune cell trafficking and surveillance (Sallusto et al., 1998). However the broad expression of CXCL12 makes it unlikely to be involved in tissue specific targeting of immune cells. CCR9 and CCL25 seem to be involved in, but not essential for, the recruitment of cells to the gut (Papadakis et al., 2000), while CCR9 and CCR10 with their ligands CCL25 and CCL28 regulate the localisation of IgA secreting B-lymphocytes to the mucosa (Hieshima et al., 2004). In contrast, CCR10+ T cells which co-express CCR4, but not B cells, home to the skin (Reiss et al., 2001). CCL17, CCL22 and CCL27, ligands for CCR4 and CCR10, are upregulated during skin inflammation but there is no evidence for a role of these chemokines during immune surveillance.

Inflammatory cytokines and chemokines are produced by sentinel cells, including dendritic cells, macrophages and γδ T cells on initiation of infection (Moser et al., 2004). Inflammatory chemokines may be induced by the pathogenic organisms themselves or cytokines including IL-1β, tumor necrosis factor (TNF)-α and interferon (IFN)-γ. Inflammatory cytokines induce the secretion of high concentrations of inflammatory chemokines by immature myeloid DCs, resulting in

<table>
<thead>
<tr>
<th>RECEPTOR</th>
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<th>LIGANDS</th>
<th>DISEASE</th>
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<td></td>
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<td>CCR1</td>
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<td>Rejection, Cancer, MS, RA, Asthma, Inflammation</td>
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<tr>
<td>CCR2</td>
<td>Me, immature DC, basophil, fibroblast, T, NK, &amp; endothelial cells</td>
<td>CCL2, CCL7, CCL8, CCL12, CCL13, CCL16</td>
<td>MS, RA, Asthma, Atherosclerosis, Rejection, Fibrosis</td>
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<tr>
<td>CCR3</td>
<td>Eosinophils, basophils, platelets, mast, Th2 and airway epithelial cells</td>
<td>CCL3, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL24, CCL26, CCL28</td>
<td>Allergy, Asthma, Atopic dermatitis</td>
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<tr>
<td>CCR5</td>
<td>DC, Mo, Th1 &amp; NK cell</td>
<td>CCL3, CCL4, CCL5, CCL8, CCL13, CCL14</td>
<td>Rejection, HIV, MS, Cancer,</td>
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<tr>
<td>CCR10</td>
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<tr>
<td>CXCR1</td>
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<td>CXCL1, CXCL6, CXCL8</td>
<td>Sepsis, Psoriasis,</td>
</tr>
<tr>
<td>CXCR2</td>
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<td>CCR8</td>
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<td>CXCL16</td>
<td>Arthritis</td>
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</tbody>
</table>

desensitisation, increased CCR7 expression and migration of these cells to the lymph nodes (Kellermann et al., 1999). In contrast plasmacytoid DCs, which are predominantly located in the lymph nodes due to expression of CXCR3 and CXCR4, respond relatively poorly to inflammatory chemokines (Penna et al., 2002). It has been proposed that secretion of CXCL12 by dendritic cells and constitutive expression of CXCL12 in the periphery may facilitate interactions between naïve T cells and these antigen-presenting cells, independent of the secondary lymphoid organs (Pablos et al., 1999). CXCL12 also enhances T cell responses to antigen stimulation through upregulation of cytokine secretion, CD40 ligand expression, proliferation and promotion of cell survival (Nanki and Lipsky, 2000, 2001; Suzuki et al., 2001). Overall the regulation of immune function is highly dependent on chemokines with these proteins regulating both the localization and function of immune cells. However our knowledge of how the chemokine system regulates immune cells is still in its infancy with many areas still remaining to be explored.

**Fuction of chemokines and chemokine receptors in disease**

**Diseases of the immune system**

One of the principal functions of chemokines and their receptors is to regulate the trafficking of immune cells throughout the body, directing them to sites of infection. Diseases of the immune system involve the inappropriate initiation, or maintenance, of inflammatory responses and result in the accumulation of immune system cells in the affected tissue. The exact composition of the immune cell infiltrate is dependent on the specific disease and is influenced by the chemokine production in the particular tissue. Due to potential redundancy of chemokines and the complex nature of immune cell recruitment to inflamed sites, dissecting and prioritising the pathways involved has been difficult. The use of antibody blockade, and gene desensitisation, increased CCR7 expression and migration of these cells to the lymph nodes (Kellermann et al., 1999). In contrast plasmacytoid DCs, which are predominantly located in the lymph nodes due to expression of CXCR3 and CXCR4, respond relatively poorly to inflammatory chemokines (Penna et al., 2002). It has been proposed that secretion of CXCL12 by dendritic cells and constitutive expression of CXCL12 in the periphery may facilitate interactions between naïve T cells and these antigen-presenting cells, independent of the secondary lymphoid organs (Pablos et al., 1999). CXCL12 also enhances T cell responses to antigen stimulation through upregulation of cytokine secretion, CD40 ligand expression, proliferation and promotion of cell survival (Nanki and Lipsky, 2000, 2001; Suzuki et al., 2001). Overall the regulation of immune function is highly dependent on chemokines with these proteins regulating both the localization and function of immune cells. However our knowledge of how the chemokine system regulates immune cells is still in its infancy with many areas still remaining to be explored.

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**Arthritis**

Rheumatoid arthritis is a chronic inflammatory disease characterized by an infiltration of Th1 cells, neutrophils and most notably monocytes in the synovial joint, resulting in cartilage destruction and bone remodeling. There are good animal models of this disease and it is probably the most extensively studied. Many chemokines and their receptors are upregulated in arthritis including CCL3, CCL4, CCL5, CCL20, CXCL8, CXCL9, CXCL10, CCR1, CCR2 and CCR5 (Hosaka et al., 1994; Robinson et al., 1995; Chabaud et al., 2001; Patel et al., 2001; Shahrara et al., 2003) and some of these chemokines have been shown to be chemo attractants for leukocytes within the synovial fluid from arthritic joints (Koch et al., 1994; Volin et al., 1998; Shaddi et al., 2002; Ruth et al., 2003). However, the importance of these chemokines in the pathogenesis of arthritis is not clear.

CXCL8 appears to be among the more important. Ectopic administration of CXCL8 in animals induces arthritis in a time and dose dependent manner, and antibody blockade of CXCL8 reduces neutrophil infiltration (Harada et al., 1994). Consistent with a role for CXCL8, patients with rheumatoid disease have high quantities of this chemokine in both synovial tissue and the fluid of affected, but not healthy joints (Endo et al., 1991; Kraan et al., 2001). Ectopic administration of CXCL2 also results in macrophage accumulation, and antibody blockade reduces disease severity in a rat model (Akahoshi et al., 1993; Ogata et al., 1997). Due to this apparent involvement of CCR2 ligands, CCR2 was thought to be important, but CCR2-/ animals were not protected from either infection or collagen induced arthritis (Brown et al., 2003; Quinones et al., 2004). Interestingly antibody-blocking experiments revealed a protective effect of CCR2 blockade during disease initiation but aggravation of the progressive stage of the disease, suggesting a possible dual function of this receptor (Bruhl et al., 2004). Blockade of CCR2 using CCL2 (9-67) reduces disease intensity, an effect that is further enhanced by the addition of CXCL1 (8-73) and blockade of CXCR2 (Gong et al., 2004). More encouragingly CXCR2-/- animals demonstrated significant protection in a collagen-induced disease model. Blockade of CXCR2 using SB225002 derivatives, reduced the intensity of acute and chronic arthritis in rabbit models (Podolfini et al., 2002), supporting a role for this receptor in arthritis (Brown et al., 2003). CCR1 and its ligands CCL3 and CCL5 may also play a role, with levels of CCL3 being directly proportional to joint pain (Tak et al., 1997) and animal studies suggesting that blockade of CCR1 or its ligands reduces joint swelling (Plater-Zyberk et al., 1997; Barnes et al., 1998). In humans an unspecified CCR1 antagonist has been evaluated in a phase I clinical trial in patients with chronic arthritis, without severe toxicity and with a significant reduction in leukocyte infiltration, including macrophages and T cells (Haringman et al., 2003).

CXCR4 may have a role in the active retention of T cells within the synovial compartment with its ligand CXCL12 expressed by synovial endothelial cells and synovial fibroblasts (Buckley et al., 2000; Nanki et al., 2000). CXCL12 also co-localizes with the sites of angiogenesis, a process it is known to modulate (Pablos et al., 2003). Inhibition of CXCR4 using the T140 derivative, TN14003, or the bicyclam AMD3100 significantly reduced joint inflammation and the severity of the disease in mouse models of arthritis (Matthys et al., 2001; Tamamura et al., 2004). Interestingly a protective role for CXCL12 has recently been reported, where CXCL12 cross desensitizes neutrophils to C5a
and fMLP induced oxidative burst, potentially revealing a more complicated scenario (Lenoir et al., 2004). Recent studies have suggested a protective influence of the CCR5Delta32 polymorphism for rheumatoid arthritis (Pokorny et al., 2004) and the CCR5 antagonist TAK-779 was effective at attenuating disease in a mouse model of arthritis (Yang et al., 2002). However, the CCR5-/- mouse is not protected from collagen-induced arthritis (Quinones et al., 2004). The localization of antigen presenting cells within the synovium is also directed by chemokines. CCL20 and CCR6 appear to be involved in the homing of immature dendritic cells to the intimal lining layer of the synovium (Page et al., 2002) and that of mature dendritic cells to the lymphocyte infiltrates by CCL19 and CCL21, the ligands for CCR7. Dendritic cells produce a number of chemokines in the arthritic synovium including CCL18, CCL19, and CCL17 (Radstake et al., 2004) while CXCL13 expression was believed to be derived from follicular dendritic cells in germinal centers of ectopic lymphoid structures frequently found in rheumatoid synovial tissue. It now appears that monocyte/macrophages may be producing this chemokine (Shi et al., 2001; Carlsen et al., 2004). The precise role of these chemokines in arthritis is not fully understood.

**Multiple sclerosis**

Multiple sclerosis is a chronic relapsing inflammatory disease with T cell and macrophage infiltrates of the perivascular regions of the central nervous system, resulting in demyelination and neuronal damage. The pathogenesis of multiple sclerosis is at least partially dependent on chemokines, with elevated levels of several chemokines being detected in the serum and/or cerebro-spinal fluid of patients with multiple sclerosis (Sorensen et al., 1999; Franciotta et al., 2001). It was thought that CCL2, associated with a Th2 response, and CCL3, associated with a Th1 response, were the most important (Karpus and Ransohoff, 1998). This was supported by experiments using CCL3 antibody neutralization and CCR1-/- mice, which demonstrated a significant degree of resistance to experimental autoimmune encephalitis (Rottman et al., 2000; Nathan, 2002). However the CCL3-/- mice showed no attenuation of the disease (Tran et al., 2000), suggesting that other CCR1 ligands may compensate for the absence of CCL3. Certainly, studies where CCL3 and CCL5 were both inhibited showed more promising results (Youssef et al., 1999). CCR1 antagonists also produced encouraging result in rodent models of multiple sclerosis, abrogating clinical and histopathological signs of disease (Eltayeb et al., 2003). The proposed role for CCR5, another CCL3 receptor, in this disease has been discounted by the studies using the CCR5-/- mice, a finding supported by the lack of protection of the CCR5Delta32 mutation in this disease (Tran et al., 2000; Silversides et al., 2004). The potential role of CCR2 in multiple sclerosis is also unclear, with CCL2-/- mice showing an altered course of the disease and the protective effect of CCR2 deletion being dependent on the strain of mice used (Fife et al., 2000; Izikson et al., 2000; Gaupp et al., 2003). Analysis of leukocytes from patients with multiple sclerosis suggests that CCL2 levels are decreased in the cerebrospinal fluid of patients with early active multiple sclerosis suggesting a limited role for this axis in the pathogenesis of multiple sclerosis (Sorensen et al., 2004).

**Asthma**

Asthma is a chronic inflammatory disease involving the accumulation of eosinophils, neutrophils and T lymphocytes in the submucosa of the small airways, as well as hyperplasia of mucus glands and subepithelial fibrosis. The inflammatory response is associated with hyper reactivity of airways, and CD4+ Th2 cells are believed to underpin the inflammatory response by regulating the accumulation of basophils, mast cells and eosinophils. Antibody neutralization experiments in animal models have suggested that multiple chemokines are involved in the recruitment of leukocytes. CCL3 and CCL5, affecting eosinophils, and CCL2, which has activity on T lymphocytes, are elevated in allergic asthma (Alam et al., 1996). Interestingly while antibody blockade of CCL2 was beneficial (Campbell et al., 1999a) the CCR2-/- mouse has exacerbated disease suggesting a complex role of CCL2 in asthma (Kim et al., 2001). In vitro studies and patient samples have most frequently implicated CCL11 and CCR3 as major players in allergic responses (Gonzalo et al., 1996; Ying et al., 1997; Campbell et al., 1999a), however the CCL11-/- mouse is only partially protected against allergic airway inflammation (Rothenberg et al., 1997; Schuh et al., 2002a) and CCR3-/- mice have yielded paradoxical results (Humbles et al., 2002). Small molecule antagonists of CCR3 including DCP-168 have apparent efficacy in murine and monkey models of asthma, however this data has not been published in mainstream literature. Antibody mediated ligand blockade of the CCL22, CCL17/CCR4 axis showed significant efficacy but the CCR4-/- mice appear to have countered the expected role of this receptor in asthma, but it does appear to be important for chronic respiratory inflammation (Chvatchko et al., 2000; Schuh et al., 2002b). After some initial positive data, the CCR8/CCL1 axis now appears to not be essential in this condition (Chung et al., 2003; Goya et al., 2003). CCR6-/- animals demonstrated reduced airway resistance, fewer eosinophils around the airway, lower levels of IL-5 in the lung, and reduced serum levels of IgE, however the lack of CCR6 expression on bronchial leukocytes makes this result difficult to understand (Lukacs et al., 2001; Liu et al., 2003). Interestingly, antibody blockade of CXCR4 decreased airway inflammation and hyper responsiveness (Gonzalo et al., 2000) and the CXCR4 antagonist AMD3100 has shown modification of disease progression in animal models.
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(Lukacs et al., 2002). Overall, there is no clear single chemokine/chemokine receptor axis that predominates the allergic inflammatory response, but it appears that it is mediated by a number of functionally and temporally overlapping activities.

Other inflammatory disorders

Psoriasis is characterised by an epidermal infiltration of neutrophils and T cells, resulting in epidermal thickening and hypertrophic papillary dermis. In apoptotic dermatitis the infiltration consists of T cells, eosinophils and dendritic cells. In both conditions CCL17 and CCL27 are displayed on the dermal endothelium, keratinocytes, dermal fibroblasts and dendritic cells (Vestergaard et al., 1999, 2000; Homey et al., 2002). These are ligands for CCR4 and CCR10 expressed on skin-homing T cells (Campbell et al., 1999b). Animal models do not faithfully mirror the human diseases but have provided some insights. Although CCR4 deficient mice do not have impaired T cell recruitment to the inflamed skin, CCL27 neutralization does impair T cell homing in wild type and CCR4-/- mice, implicating CCR10 (Reiss et al., 2001; Homey et al., 2002). Other chemokine receptors and their ligands implied in these diseases include CXCRI3, and ligands CXCL9, CXCL10 and CXCL11, CCR6 and CCL20, and CCR3 and CCL11 (Gerber et al., 1997; Yawalkar et al., 1999; Homey et al., 2000; Flier et al., 2001).

Crohn’s disease and ulcerative colitis are characterised by mixed inflammatory cell infiltration of the bowel mucosa. Crohn’s disease predominantly involves a Th1 type response, while ulcerative colitis is Th2 type (Farrell and Peppercorn, 2002; Shanahan, 2002). Chemokines implicated in these diseases include CXCL8, CXCL5, CCL2, CCL11, CXCL10 and CX3CL1 (D’Ambrosio et al., 2003). CCL25 and CCR9 have been implicated in the recruitment to the small bowel but not to the colon in Crohn’s disease (Gerber et al., 1997; Papadakis et al., 2001). Consistent with Th1 involvement, CXCR3 is expressed on infiltrating T cells in Crohn’s disease, while CCR3 is found in ulcerative colitis (Yuan et al., 2001). CCR2 and CCR5-/- mice are protected from colitis (Andres et al., 2000) but CCR5Δ32 does not protect against this disease in humans (Martin et al., 2001).

Graft rejection

Until recently allograft rejection was largely considered to be simply the result of antigen presentation. However antigen-independent injury to the grafted tissue contributes to the initial recruitment of inflammatory cells to the graft and is most commonly due to ischemia-reperfusion injury. Furthermore, brain death of donors leads to increased inflammatory chemokine production in donor tissues and is associated with increased ischemia-reperfusion injury, and increased acute and chronic rejection, resulting in reduced graft survival (Terasaki et al., 1995; Kusaka et al., 2000; Wilhelm et al., 2000; Pratschke et al., 2001). The mediators of neutrophil recruitment are tissue specific but the main contributors are CCL3, MIP-2 (murine homologue of CXCL2&3) and KC (murine homologue of CXCL1) in rodents and CXCL8 in humans. The resulting inflammation induces the production of other chemokines, such as CCL2 and CXCL10, which attract macrophages and apparently antigen unprimed T cells respectively, resulting in acute rejection (Hancock et al., 2001; El-Sawy et al., 2004). Recruited activated T cells then produce CCR5 ligands which consolidate the antigen dependent immune response (Koga et al., 1999). The mechanisms underlying the establishment of chronic rejection appear to be varied and are less well understood but involve graft atherosclerosis. This is most likely induced by antigen dependent vessel damage or injury resulting from immunosuppressive agents and exacerbated by transplant unrelated risk factors (Hancock, 2003). Overall CXCR3 and its ligand CXCL10 appear to play the most prominent role in allograft rejection. CXCR3 and CXCL10 expression are associated with rejection in renal, lung, liver and cardiac allografts in humans (Agostini et al., 2001; Goddard et al., 2001; Melter et al., 2001; Segerer et al., 2001). CXCR3-deficient recipients and recipients of grafts from CXCL10-deficient donors both experience prolonged graft survival which can be translated to graft acceptance when combined with sub therapeutic doses of immunosuppression in CXCR3-/- recipients (Hancock et al., 2000, 2001; Baker et al., 2003). CXCL9, another CXCR3 ligand has also been shown to play a role in rejection of skin and lung grafts using antibody depletion experiments (Belperio et al., 2003). However CXCR3 and CXCL9 have only a marginal role in vascular occlusion seen in chronic rejection (Yun et al., 2002). CCR5 and CCR1 are also of major importance, with deletion of either resulting in enhanced graft survival and completely preventing chronic rejection (Gao et al., 2000, 2001). The CCR1 antagonists BX471 and met-RANTES have been shown to attenuate graft rejection in animal models of heart and kidney transplantation, an effect that was further enhanced by combination with the immunosuppressive drug cyclosporin (Horuk et al., 2001a,b; Yun et al., 2004). The role of CCR5 is further supported by the enhanced survival of grafts in recipients homozygous for CCR5Δ32 (Fischeder et al., 2001). Redundancy of chemokine ligands is clear from the lack of effect of deletion of any single CCR5 ligand (Gao et al., 2001). CCR7 appears to play a supporting role with CCR7-/- animals showing only a mild extension in graft survival times (Beckmann et al., 2004).

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease where leukocytes, particularly monocytes/macrophages,
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Infiltrating the vessel wall play a pivotal role. Aberrant chemokine expression within the vessel wall and chemokine receptor expression on leukocytes significantly contribute to the abnormal leukocyte infiltration. The principal chemokines involved in atherosclerosis are CCL2, CXCL8 and CX3CL1. CCL2 is present in atherosclerotic plaques in humans and rodents, and its receptor, CCR2, is upregulated on monocytes by hypercholesterolemia (Han et al., 1998; Terkeltaub et al., 1998). CCR2 appears to be involved in the initial entry of monocyte/macrophages into the vessel wall, with CCR2-/- atherosclerosis prone mice having reduced lesion size, with reduced macrophages than wild type mice (Boring et al., 1998; Gu et al., 1998; Gosling et al., 1999). CCR2 also enhances the migration/proliferation of smooth muscle cells with CCR2-/- animals having reduced intimal thickening following vessel injury (Roque et al., 2002). Inhibition of CCL2 by gene therapy in rodents attenuated both the initiation and progression of atherosclerotic plaques, further underpinning a significant role for the CCL2/CCR2 axis in this disease (Ni et al., 2001; Inoue et al., 2002).

Initially, evidence for CX3CR1 in atherosclerosis came from epidemiological studies where polymorphisms, later found to impart reduced function, were associated with a lower risk of coronary artery disease (Moatti et al., 2001; McDermott et al., 2003). CX3CR1 is expressed on circulating monocytes and smooth muscle cells in human atherosclerotic plaques (Lucas et al., 2003). CX3CL1 is present in atherosclerotic arteries but the source of the chemokine is controversial (Wong et al., 2002; Lesnik et al., 2003). However CX3CR1-/- mice have reduced disease as measured by a number of parameters (Combadiere et al., 2003; Lesnik et al., 2003).

CXCR2 is also expressed on macrophages and its expression enhances their accumulation in plaques under the influence of CXCL8/CXCL1, although the underlying mechanisms and relative importance of this axis are not known (Boisvert et al., 1998). CCR5 deficiency is not protective in the early stages of atherogenesis in apoE knockout mice, excluding this axis from playing a major role (Kuziel et al., 2003). Other chemokines implicated in this disease include CCL11, due to a recent association of a polymorphism in this gene with an increased risk of myocardial infarction, (Zee et al., 2004) and CXCL16 and a novel human CC chemokine, leukotactin-1, due to elevated expression in atherosclerotic lesions (Wuttge et al., 2004; Yu et al., 2004).

Infection

During infection immune cell infiltration and inflammation is normally appropriate. However, prolonged inflammation can contribute to the morbidity and mortality resulting from acute viral infection. Some examples of where this can occur include infections with respiratory syncytial virus, and influenza virus, and severe acute-respiratory syndrome (Kuiken et al., 2003; Nicholls et al., 2003). CCR1 plays a major role in maintaining the inflammatory response to a variety of infectious agents, a role confirmed by the phenotype of the CCR1-/- knockout mice (Gao et al., 1997; Blease et al., 2000; Khan et al., 2001). Consistent with this the CCR1 antagonist, Met-RANTES improves outcome in animal models of viral infection (Bonville et al., 2004). A similar phenotype was also observed for CCR2-/- animals (Kurihara et al., 1997; Warmington et al., 1999; Blease et al., 2000; Sato et al., 2000). In respiratory syncytial virus and influenza virus infections, the predominant chemokine mediating prolonged inflammatory responses appears to be CCL3 with reduced responses to influenza virus being observed in CCL3/-/- mice translating into enhanced survival (Cook et al., 1995; Bonville et al., 2004).

Summary

Although progress has been made in understanding which chemokines/receptors are important in specific inflammatory diseases there is still a long way to go before this is well understood. Knockout animal models have at times corroborated results from experiments with blockade of chemokines or their receptors using antibodies or specific antagonists. However at times the gene knockout animals have produced surprising results which conflict with preceding data. There are a number of potential explanations for such experimental disparities including: compensation for the deleted gene in knock out animals by other chemokines or their receptors; and deletion of cell subsets, not related to chemokine receptor function, in antibody blockade experiments. Extrapolating results obtained using animal models to applications in human disease needs to be made with caution. Differences between the murine and human immune systems exist (Mestas and Hughes, 2004), and promising results in animal models have failed to fulfill their promise in the clinic.

Viral entry of HIV

HIV entry into cells requires the fusion of its viral envelope with the cell membrane. This normally occurs through an initial binding of the viral envelope protein, gp120, to CD4, followed by a second interaction with a co-receptor, primarily the chemokine receptors CXCR4 or CCR5 (Greene and Peterlin, 2002). HIV strains are mostly restricted to using CCR5 (R5-tropic) or CXCR4 (X4-tropic) although some strains can utilise both co-receptors (Alkhatib et al., 1996; Dragic et al., 1996; Feng et al., 1996; Berger et al., 1998). R5 viruses are usually transmitted between individuals and infect both macrophages and primary T cells, while X4 strains develop in about 50% of infected individuals and are usually associated with disease progression (Connor et al., 1997). X4 strains infect T cells and T cell lines but
not macrophages (Deng et al., 1996). Individuals homozygous for defective CCR5A32 are highly resistant to HIV infection, underscoring the importance of this receptor in HIV transmission (Huang et al., 1996; Michael et al., 1997). As a result, inhibitors of coreceptor engagement have been intensively investigated as therapeutics for HIV infection.

A number of antagonists have proved effective in animal models including the CCR5 antagonist SCH-C tested in NOD/SCID mice xenografted with human liver and thymus and AK602 (ONO4128/GW873140) (Maeda et al., 2003). In clinical trials the CCR5 antagonists UK-427,857, SCH-C and a newer compound SCH-D significantly reduced viral loads (Reynes et al., 2002; Abel et al., 2003; Napier et al., 2003; Pozniak et al., 2003; Schurmann et al., 2004). CXCR4 antagonists ALX40-4C, AMD3100, AMD070 and KRH-1636 inhibited in vitro viral replication of X4 strains in the nanomolar range (Doranz et al., 1999; Schols et al., 1997; Ichiyama et al., 2003) and, in phase II clinical trials, AMD3100 treatment resulted in significant reduction in viral loads in patients harbouring X4 strains (Schols et al., 2002). Although these results are promising there have continued to be toxicity issues related to doses of the drugs required for therapeutic effect. New agents are continually being generated with improved efficacy and reduced toxicity making it possible that these agents may eventually be useful therapeutic for the treatment and possibly the prevention of transmission of HIV (Lederman et al., 2004).

However a number of other hurdles remain. HIV is a highly polymorphic virus, existing as number of genetically related variants or quasispecies which are subject to rapid evolution (Meyerhans et al., 1989; Domingo, 1992; Holland et al., 1992). Previous experience with other anti-HIV agents has shown that the highly polymorphic nature of the virus is a primary stumbling block to achieving control of disease progression (Mansky, 2002). Switching of viral strains from X4 to R5 and visa versa has already been observed in the presence of specific inhibitors (Este et al., 1999; Mosier et al., 1999). Of more concern is the emergence of viral strains resistant to antagonists in the absence of altered co-receptor usage (Schols et al., 1998; Kanbara et al., 2001; Trkola et al., 2002). Encouragingly these emerging resistant strains appear to have reduced replicative and pathogenic potential (Armand-Ugon et al., 2003).

Cancer cell biology

Chemokines have been shown to be involved in all stages of cancer development including neoplastic transformation, tumor growth, immune evasion, metastasis and angiogenesis. As a result there has been great interest in understanding how chemokines and their receptors influence cancer cell behaviour, with the aim of developing new therapeutic strategies. Altered expression of chemokines and/or their receptors has been reported in a large number of malignancies, although the reasons underlying this are not known.

CXCR4 expression and function is increased by NFκB in breast cancer cells (Helbig et al., 2003). NFκB is frequently constitutively active in malignant cells. Staller et al (Staller et al., 2003) also demonstrated that loss of the tumor suppressor gene von Hippel-Lindau resulted in upregulation of CXCR4 under the hypoxic conditions common within tumors. In addition VEGF and oestrogen can upregulate expression of CXCR4 in breast and ovarian tumors respectively (Bachelder et al., 2002; Hall and Korach, 2003). An example of the role of chemokine receptors in neoplastic transformation is the herpesvirus-8 encoded G protein coupled receptor ORF74. It is homologous to CXCR2 and although able to signal independently of ligand, the CXCR2 ligands CXCL1 and CXCL8 further stimulate signalling through this receptor. Over expression of this receptor in the hematopoietic compartment of transgenic animals results in angioproliferative lesions reminiscent of Kaposi’s sarcoma (Yang et al., 2000).

Proliferation

A number of chemokines have been identified as regulating the growth of various malignancies. CXCL8 can act as an autocrine growth factor in melanoma, colon, gastric, hepatic and pancreatic cancer cell lines (Schadendorf et al., 1993; Miyamoto et al., 1998; Brew et al., 2000; Fujisawa et al., 2000; Takamori et al., 2000) and the expression of both CXCL8 and its receptor CXCR2 in neuroblastoma, ovarian carcinoma and squamous cell carcinoma of the head and neck suggest that it may also play a role in these malignancies (Richards et al., 1997; Ferrer et al., 2000; Ivarsson et al., 2000). Other CXCR2 ligands, including CXCL1, CXCL2 and CXCL3, have also been implicated in the growth of melanomas, adenocarcinomas, gastric, lung and pancreatic cancers (Richmond and Thomas, 1986; Owen et al., 1997). More recently CXCR4 has been reported to be over-expressed in a number of malignant cell types including breast (Muller et al., 2001), prostate (Sun et al., 2002), renal (Staller et al., 2003), pancreatic (Kohshiba et al., 2000), glioblastoma (Rempel et al., 2000), lung (Kijima et al., 2002) B cell chronic lymphocytic leukemia (Barretina et al., 2003), and autocrine growth mediated through this receptor demonstrated in glioblastomas (Sehgal et al., 1998; Barbero et al., 2003). CXCL12 has also been implicated in the growth of colon cancers (Sebolt-Leopold et al., 1999), chronic B cell leukemia (Burger et al., 2000) and acute lymphoblastic leukemia (Nishii et al., 1999; Juarez et al., 2003), non-Hodgkins lymphoma (Bertolini et al., 2002), small cell lung cancer (Kijima et al., 2002), glioma (Zhou et al., 2002; Barbero et al., 2003) and breast and ovarian cancer (Scotton et al., 2001; Hall and Korach, 2003).
Metastasis

The fatal nature of cancer is highly related to the ability to spread from the original site and establish secondary tumors in distant parts of the body. The process of metastasis requires the acquisition of a number of features including the ability of cells to detach from the bulk of the tumor, transmigrate through the extracellular matrix, penetrate the cellular layers of blood and lymphatic vessels, move through tissue and survive while detached. Chemokines and their receptors have been implicated in a number of these processes. CXCL8 can enhance the transcription of metalloproteinase (MMP)-2 in melanoma cells (Luca et al., 1997) and over-expression of CXCL8 increases MMP-9 production in prostate cancers, resulting in increased local invasion of tumors in nude mice (Inoue et al., 2000). The activator of MMP-2, MT1-MMP, is upregulated by CXCL12, resulting in increased invasion of basement membrane (Bartolome et al., 2004). Chemokines are classically known as chemotactants and have recently been implicated in the non-random pattern of metastasis displayed by tumors and indeed metastatic patterns of various tumor types can be theoretically explained in some instances by the expression of chemokine receptors by the tumor and ligands by the preferred sites of metastases. The best-studied system is the CXCR4/CXCL12 axis. Muller et al. demonstrated that CXCL12 induced actin polymerisation, pseudopodia formation, chemotaxis and invasive responses in the breast cancer cell line MDA-MB-231 (Muller et al., 2001). These findings have been supported by in vitro studies of the role of CXCL12/CXCR4 in the metastatic process in different cancers, including breast, ovarian, lung and prostate (Koshiba et al., 2000; Bachelder et al., 2002; Scotton et al., 2002; Taichman et al., 2002; Sun et al., 2003). In murine models of metastasis, monoclonal antibodies blocking CXCR4 function or intrakine-mediated down modulation of surface expression of this receptor have demonstrated reduced metastasis in breast cancer, Non-Hodgkin’s lymphoma, T cell lymphoma, non-small-cell lung carcinoma and melanoma (Muller et al., 2001; Zeelenberg et al., 2001; Bertolini et al., 2002; Phillips et al., 2003). The CXCR4 antagonist T140 and its newer analogue TN14003 have similarly inhibited breast cancer metastasis (Murakami et al., 2002; Liang et al., 2004), while the related peptide, T22, inhibited lung metastasis of murine B16 melanoma cells, and TN14003 lung metastasis of melanoma cells (Tamamura et al., 2003). It has also been suggested that CXCR4 may facilitate metastasis by enhancing tumor cell adhesion to vascular endothelium, as is observed for hematopoietic cells (Murakami et al., 2002). However it is not entirely clear whether the CXCR4/CXCL12 axis is responsible for the seeding of metastases or the growth of established metastases at sites where CXCL12 is expressed, since CXCR4 deficient colon carcinoma cells seeded the lungs with a similar frequency as their CXCR4 expressing counterparts but the metastases failed to grow (Zeelenberg et al., 2003). Similarly the small molecule CXCR4 antagonist, AMD3100, inhibited growth of glioblastoma and medulloblastoma xenografts (Rubin et al., 2003). Surprisingly, AMD3100 reduced growth of lymphomas in vivo by 40-60% when administered thrice weekly but enhanced tumor growth when given by continuous infusion (Paul et al., 2002). Other chemokine receptors have been similarly implicated in the metastatic process. CCR7 is expressed by breast (Muller et al., 2001), gastric (Mashino et al., 2002), non-small-cell lung (Takanami, 2003), head and neck (Wang et al., 2004), and oesophageal squamous carcinomas (Ding et al., 2003) and chronic lymphocytic leukaemia (Till et al., 2002), and its expression associated with metastatic potential and poor outcome. The CCR7 ligand, CCR21, is expressed in the lymph nodes to which these cancers frequently spread. In addition CCR3 and CCR4 have been implicated in skin infiltration of malignant T cells (Ishida et al., 2003; Kleinhans et al., 2003), CCR10 in skin metastases (Muller et al., 2001) and CXCR3 in lymph node metastases in melanoma (Kawada et al., 2004).

Angiogenesis

Angiogenesis is required for tumor growth beyond 2mm diameter. A number of chemokines have stimulatory roles in angiogenesis including CXCL1, CXCL2, CXCL3, CXCL8 and CXCL12 (Gupta et al., 1998). CXCL8 levels are elevated in the serum of patients with prostate cancer and antibodies to this chemokine can reduce tumor growth in a NOD/SCID model of this disease (Moore et al., 1999; Veltri et al., 1999). Over-expression of CXCL1, CXCL2 or CXCL3 in melanocytes results in highly vascularised tumors (Luan et al., 1997). Additionally, CXCL12 expression in regions of angiogenesis immediately adjacent to areas of necrosis suggests that malignant cells utilise the angiogenic properties of CXCL12 to facilitate further vessel formation to sustain tumour growth (Rempel et al., 2000).

Immune response

Tumors not only consist of malignant cells but also contain a range of immune cells. The composition of these cells can influence the nature of the immune response elicited against the tumor. The chemokines present within the tumor directly influence the composition of tumor-associated and infiltrating leukocytes. In epithelial ovarian cancer the level of CCR5 correlates with the number of tumor infiltrating CD8+ thymocytes and with total T cell numbers in the ascites (Negus et al., 1997; Miliken et al., 2002), while in mice bearing breast tumors the CCL5 antagonist MET-RANTES produced modest inhibition of tumor growth, which was associated with reduced leukocyte infiltrate (Robinson et al., 2003). However, the extent of
leukocyte infiltration is not directly predictive of outcome. The ability of lymphocytes to kill tumor cells, and thereby regulate tumor growth, can also vary extensively. In non-small-cell lung carcinoma CCL5 expression was associated with increased lymphocyte infiltrate and was a positive predictor of survival (Moran et al., 2002). In Hodgkin’s lymphoma cytokines and chemokines produced by the tumor cells invoke a strong Th2 response, which antagonises the Th1 response which is needed for effective killing of tumor and virally infected cells (Skinnider and Mak, 2002). In breast cancer and oesophageal squamous-cell carcinoma high levels of CCL2 are associated with a high infiltration of macrophages, a poor prognosis and tumor cell invasiveness respectively (Luboshtis et al., 1999; Saji et al., 2001; Ohta et al., 2002). In contrast this same chemokine results in improved survival in patients with pancreatic cancer, an effect thought to be mediated by increased inflammation (Monti et al., 2003).

CXCL12 may also contribute to tumour immune evasion through recruitment of cells that negatively regulate the cellular immune response. Zou et al demonstrated that CXCL12 is a chemoattractant and a survival factor for plasmacytoid precursor dendritic cells associated with epithelial ovarian carcinomas (Zou et al., 2001). These cells express low levels of T-cell co-stimulatory molecules, are poor activators of naïve T-cells, and were found to elicit IL-10 production which inhibited T-cell proliferation and IFNγ production. In contrast other groups have reported a positive immunomodulatory activity for CXCL12 when transduced in various tumour cells (Nomura et al., 2001; Dunussi-Joannopoulos et al., 2002). This disparity highlights the complex role of the CXCL12/CXCR4 axis in tumorigenesis and the need for further understanding of its role in normal physiology and pathogenesis in order for therapeutic interventions to exact a positive anti-tumour effect.

Conclusion

Chemokines and their receptors obviously play significant roles in normal physiology and the pathophysiology of a number of disease states. This provides the opportunity to develop new therapeutic strategies to treat such diseases. However the complex and often redundant roles of chemokines in disease pathophysiology represents a major challenge. Specific targeting of the relevant chemokine/receptors for each condition will be necessary for significant therapeutic benefit in the absence of unacceptable toxicity resulting from the disruption of the normal physiological role of these proteins. Conditions such as HIV infection where essentially only two chemokine receptors are involved represents a simpler system for therapeutic intervention. However even here viral evolution and the development of resistance represent potential stumbling blocks. Many conditions where chemokines play prominent role are chronic requiring long term blockade of chemokine/receptor interactions. This has major implications regarding the toxicity and drug scheduling. The exceptions to this include acute infection and the initial inflammatory response resulting from ischemia reperfusion injury in organ transplantation. Although disruption of chemokine signalling has great therapeutic potential there are large gaps in our knowledge of the basic biology of these agents is health and disease. Without doubt some blockade of chemokine signalling will be used for the treatment of disease however how best to target, and indeed which networks to target is yet to be elucidated.

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