Chemokine receptors in tissue cells and angiogenesis

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Introduction

Although chemokines have been initially discovered and universally known as cytokines able to recruit leukocytes to inflamed tissues (chemotactic cytokines) and, therefore, to play an important role in the context of the immune response, subsequent studies have clearly shown that they also act on several other cell types, thus behaving as multifunctional mediators. The nature and classification of chemokines, their receptors and signalling pathways, as well as their activity of recruitment on the cells of the immune system have been discussed in other chapters of this book. Here, therefore, we will concentrate on the production of chemokines by, and on their functional activity on, tissue cells, and we will particularly focus on the essential role of chemokines on the induction and control of angiogenesis.

Chemokines in embryogenesis

Cell migration is an integral component of embryogenesis, particularly since cell position is a primary determinant of cell fate. Not surprisingly, there are complex arrays of regulators, which direct cell movement by modulating adhesion, attraction, and repulsion. Several chemokine receptors have been found to be expressed in the mouse embryo, the message encoding CXCR4 being the predominant chemokine receptor detected [1]. CXCR4- and CXCL12-deficient mice [2, 3] showed defects in the development of neuronal, cardiac, vascular, haemopoietic and craniofacial systems. Other chemokine receptor messages were also found, but all of them concordant temporally and spatially with definitive (adult-like) haematopoiesis. CX3CL1, CXCL10 and CXCL12 are certainly involved in the development of human kidney, CX3CL1 being strongly expressed during glomerulogenesis, while CXCL10 and CXCL12 in developing kidneys were more limited to focal expression [4]. More recently, CXCL12 has been found to play an essential role in promoting primordial germ cell transmigration through epithelial-like struc-

Chemokine Biology – Basic Research and Clinical Application, Volume I
edited by Bernhard Moser, Gordon L. Letts and Kuldeep Neote

tures, such as the hindgut epithelium in mouse and the endothelium in chick [5]. Of note, a possible role of interactions between CCR1 and its ligands in the initiation of trophoblastic invasion of maternal tissue has also been suggested [6]. The important role of chemokines in embryogenesis control represented the first evidence that chemokine receptors might also be expressed by resident cells in different tissues. Indeed, a large converging evidence has recognised the pivotal role of chemokines and their receptors in the biology of resident tissue cells largely beyond their chemotactic properties.

Chemokine receptors in epithelial tissues

Although chemokines were originally defined as host defense proteins and their main role is leukocyte recruitment, they and their receptors have other biological actions. Furthermore, many environmental stimuli of host of pathogen origin may lead to the induction of inflammatory chemokines expression and production in tissue cell types.

The expression of multiple chemokines in inflamed tissues, such as in the synovial lining cells of rheumatoid joints [7], autoimmune lesions in multiple sclerosis [8], ulcerative colitis and Crohn's disease [9], lung inflammation [10], sarcoidosis [11] and asthma [12], and the vascular inflammation that characterises arteriosclerosis [13], is well documented. Several receptors for inflammatory chemokines, CCR1, CCR2, CCR5 and CXCR3 in particular, are regularly detected in such lesions, while the expression of CCR3 tends to be restricted to allergic pathologies and the IL-8 receptors, CXCR1 and CXCR2, are more frequent in acute inflammation.

However, a great number of *in vivo* and *in vitro* studies demonstrated also the constitutive expression of chemokine receptors by resident epithelial cells of different tissues. The pattern of chemokines and chemokine receptors expression in epithelial tissues is summarised in Table 1.

Chemokines affecting vasculature-associated pericytes

Several studies have shown that the pericytes, smooth muscle-like mural cells that coat the wall of microvessels and are responsible for tissue fibrosis, may both express chemokines and be targets of the chemokine action [27–30]. In fact, pericytes express chemokine receptors, which, upon activation, elicit biologic actions that favour the processes of wound healing, including proliferation, migration, and extracellular matrix synthesis [31–33].

Human vascular smooth muscle cells (SMCs) express CCR2 [34], which makes these cells a likely target for CCL2. In fact, CCL2 can enhance the expression of

Table 1 - Expression and function of chemokine receptors in epithelial tissue cells

Type of tissue cells	Chemokine receptors	Function
Keratinocytes	CCR3 [14]	Inflammatory modulation
	CXCR1/CXCR2 [15]	Chemotaxis and proliferation
	CXCR3 [16]	Chemotaxis
Bronchial epithelial cells	CCR2 [17]	Proliferation and healing
	CCR3 [18]	Epithelial cell migration and proliferation
	CXCR4 [19]	Inflammatory modulation
Intestinal epithelium	CCR5 [20]	Cell migration
	CCR6 [21]	Cell migration, maintenance and renewal of the epithelium
	CXCR4 [20]	Hepatocytes
Ductular epithelial cell	CXCR4 [22]	Apoptosis
	CX3CR1 [23]	Wound healing response
Ectocervical epithelial cells	CCR5 [24]	Potential targets of HIV-1 infection
Podocyte	CCR4, CCR8, CCR9, [25]	Release of oxygen radicals
	CCR10, CXCR1-CXCR5 [25]	Release of oxygen radicals
	CXCR3 [26]	Induction of nephrin and podocin

integrins [35] as well as tissue factor [36] on SMCs. More recent findings [37] suggest that CCL2 can also directly induce SMC proliferation by stimulating the binding activity of activator protein 1. Cultured human arterial SMCs possess CCR5 at both mRNA and protein levels [33]. CCR5 on SMCs is functionally coupled, responding to CCL4 with increases in intracellular calcium concentration and tissue factor activity. CCR5 and CCL4 were also detected in SMCs of the atherosclerotic arterial wall, where they may play a role in mediating the inflammatory and prothrombotic responses associated with atherosclerosis. On the contrary, as determined by RT-PCR, human aortic SMCs do not express mRNA for other CCRs, including CCR1 [38], CCR3 [39], CCR4 [40], and DARC [41]. CXCL10 has been shown to act as a mitogen and chemoattractant for SMCs. Moreover, SMCs express CXCL10 in response to IL-1β and TNF-α in conjunction with IFN-γ and also in response to vascular injury, suggesting a role in pathogenesis of vascular diseases and injury [28].

Hepatic stellate cells (HSCs) and glomerular mesangial cells (MCs) are tissue-specific pericytes involved in tissue repair, a process that is regulated by chemokines.

In MCs expression of CCL2, CCL5, CXCL8, and CXCL10 has been repeatedly demonstrated [41–49]. CCL2 is rapidly upregulated in mouse, rat and human MCs after their activation by a variety of stimuli [41–43]. CCL5 is expressed 2 h after TNF-α stimulation by mouse MC [44] and it is also found to be expressed by primary human MCs [45]. CXCL8 is expressed by rat and human MCs [46, 47] and the expression of CXCL10 mRNA has been described for both mouse and human MCs [48, 49].

The expression of the chemokine receptor CXCR3 on human MCs was first reported by Romagnani P. and colleagues [31]. High expression of this receptor by MCs was seen by immunohistochemistry in kidney biopsies from patients with glomerulonephritis, characterised by resident mesangial cell proliferation, such as IgA nephropathy, membranoproliferative glomerulonephritis or rapidly progressive glomerulonephritis (also defined as "proliferative glomerulonephrites"). Moreover, CXCR3 was also found on the surface of cultured human MC (HMC), and appeared to mediate both intracellular Ca^{2+} influx and cell proliferation [50]. Furthermore, it was found that in both HMC and other types of vascular pericytes, CXCL10 and CXCL9 also induce chemotaxis and CXCR3 triggering results in Src activation, which in turn leads to the recruitment of Ras and activation of the ERK cascade [50]. In parallel, activation of PI 3-K and Akt can also be observed [50]. Taken all together, these findings may account for at least some mechanisms involved in the pathogenesis of proliferative GN.

Constitutive expression of the chemokine CCL21 on human podocytes and of its corresponding receptor CCR7 on MCs was also shown by immunohistochemistry of human kidney and these findings were confirmed in cultured cells and isolated glomeruli [51]. CCL21 has a positive effect on the proliferation and migration of MCs and leads to increased cell survival in Fas-induced apoptosis of human MC [51]. Moreover, activation of CCR7 on MCs by CCL21 enhances the degree and firmness of cell adhesion and increases cell spreading and the formation of cell–cell contacts, including integrin-linked kinase activation and F-actin rearrangements [52].

Inducible expression of the chemokine receptor CCR1 by human MCs after stimulation with a combination of the proinflammatory cytokines TNF-α, IL-1β and IFN-γ, has also been described [32]. In contrast to the effects observed with the ligands for CCR7 and CXCR3, stimulation of MCs with the CCR1 ligand CCL5 had no effect on cell proliferation and apoptosis. In conclusion, local chemokine generation and chemokine receptor expression on MCs may play an important role in the maintenance of glomerular homeostasis and in local remodelling processes.

HSCs express and secrete several CC chemokines, including CCL2 and CCL3 [53, 54]. Several lines of evidence indicate that CCL2 plays a role in the recruitment and maintenance of the inflammatory infiltrate during liver injury. CCL2 secretion is upregulated during chronic hepatitis and correlates with the number of cells infiltrating the portal tract [55]. *In vitro* and *in vivo* data indicate that HSCs may con-

tribute to the expression of CCL2 within the liver during both chronic and acute injury [53, 54, 56]. On cultured human HSCs, CCL2 stimulates migration in a dose-dependent fashion and activates intracellular signalling, such as increase in cytosolic calcium concentration, PI3-K activity, protein tyrosine phosphorylation [56]. Cultured HSCs express functional CCR7, the activation of which stimulates cell migration and accelerates wound healing in an *in vitro* model. Exposure of HSCs to CCL21 triggered several signalling pathways, including extracellular signal-regulated kinase, Akt, and nuclear factor κB, resulting in induction of proinflammatory genes [57]. HSCs express CCR5, as shown by flow-cytometric analysis and RT-PCR [57], and respond to CCL5 with an increase in both intracellular calcium concentration and free radical formation. Furthermore, CCL5 induced ERK phosphorylation and HSC proliferation. Additionally, CCL5 induced focal adhesion kinase phosphorylation and a substantial increase in HSC migration [58]. HSC expressed functional CXCR3 receptors on the cells surface, and interaction with CXCR3 ligands resulted in increased chemotaxis, but not proliferation, through the Ras/ERK signalling cascade. Activation of CXCR3 stimulated Src phosphorylation and kinase activity and increased the activity of PI3-K [50].

Chemokines control of angiogenesis and wound healing

Tissue repair

Models of skin wound healing mimic inflammatory reactions that might also be relevant to infectious processes in general [59]. In this model, the interplay of CXC chemokines with growth factors, cytokines and adhesion molecules not only influences the sequential participation of inflammatory cells but, more importantly, regulates the inflammatory reaction leading to angiogenesis, tissue repair and new tissue generation [59, 60]. The repair process is initiated immediately after injury of blood vessels through the release from degranulating platelets of various growth factors, such as vascular endothelial growth factor (VEGF)-A, platelet-derived growth factor (PDGF), and several chemokines in large quantities. CXCL1, CXCL5 and CXCL7 initiate the neutrophil recruitment [59, 61, 62], whereas high amounts of CXCL4 contribute to the formation of blood clots [63]. This provides a barrier against invading microorganisms and serves as a matrix for the attachment of inflammatory cells that are recruited to wound tissue within a few hours of injury. The initial vessel-associated expression of CXCL1 facilitates neutrophil diapedesis [64]. Subsequently, the cooperative expression of CXCL1 and CXCL8 in the superficial wound bed supports additional neutrophil migration to the wound surface [65]. Neutrophils produce a wide variety of proteinases and reactive oxygen species as a defense against contaminating microorganisms and they are involved in the phagocytosis of cell debris. CXCR2 is also expressed on neovascularising ECs [65].

The time course of CXCL8 expression correlates with massive angiogenesis between days 1–4 [64], leading to the formation of new blood vessels. The newly formed connective tissue is known as granulation tissue because of the granular appearance of several capillaries. Accordingly, *CXCR2*-deficient mice exhibit a defective neutrophil recruitment, delayed monocyte recruitment and severe impairment of angiogenesis at the site of wounding [66]. Neutrophil accumulation is followed by the immigration of monocytes and macrophages, as a result of CCL2/CCR2 chemokine system [64, 67]. Interestingly, from days 0–6 after wounding, CXCL12 production by keratinocytes and fibroblasts is progressively downregulated, because of the inhibitory effect exerted by IL-1 and TNF. Given the ubiquitous expression of CXCR4 on both resident and inflammatory cell types, this probably represents a counter regulatory mechanism to avoid chronic inflammation [68]. High numbers of lymphocytes are also recruited during the whole period of healing and they represent the major leukocyte subpopulation on day 14. Between days 1–4, CXCL11, which is constitutively produced on the surface of human microvascular endothelial cells (HMVECs) [60] and is highly induced by epithelial monolayer disruption [64], contributes to the pronounced lymphocyte accumulation. Subsequently, CXCL9 and CXCL10, which are both T cell attractants [69, 70], are highly expressed at sites of lymphocyte accumulation [64]. Indeed, activated lymphocytes express high levels of CXCR3 [71]. The fact that vascularity increases until day 4, but remains constant afterwards, despite the presence of growth factors, such as bFGF and PDGF, suggests that the angiostatic properties of CXCL9 and CXCL10 can prevent unlimited vessel growth. In this context, the cell cycle dependence of CXCR3-B expression by HMVECs is of crucial importance [71]. Indeed, only 'angiogenic' ECs can respond to angiostatic stimuli, and therefore they arrest both migration and growth through inhibition mediated by CXCL11 present on the surface of adjacent ECs. This mechanism enables the generation of a finely regulated network of vessels (see below) without altering the properties and functions of quiescent ECs, which cannot respond to angiostatic chemokines. Finally, CXCL10, CXCL9 and CXCL11 mediate the migration of CXCR3-A-expressing pericytes and their proliferation around nascent vessels. The opposite effects of CXCL9, CXCL10 and CXCL11 on ECs and pericytes could be explained by distinct and sequential steps leading to angiogenesis. Of note, recruitment of pericytes occurs after the progression phase of angiogenesis that is determined by EC positioning and proliferation. The association of pericytes to newly formed blood vessels has been suggested to regulate endothelial cell proliferation, survival, migration, differentiation, and vascular branching. Therefore, these chemokines could contribute to vessel stabilisation by inhibiting cell cycle progression in ECs.

Migration and proliferation of keratinocytes at the wound edge are followed by the recruitment and proliferation of dermal fibroblasts. These cells subsequently acquire a contractile phenotype and transform into myofibroblasts, which have a major role in wound contraction. CXCL8 might directly stimulate re-epithelialisa-

tion, as a result of stimulating keratinocyte proliferation [72]. However, wound contraction is diminished by topical application of CXCL8, suggesting that elevated levels of this chemokine might also contribute to retarded wound repair [73]. Finally, a transition from granulation tissue to mature scar occurs, which is characterised by continued collagen synthesis and catabolism. CXCL10 and CXCL11 also deliver signals to the dermal compartment to synchronise the re-epithelialisation process. Indeed, these chemokines limit EGF-induced fibroblast motility, but promote the chemotaxis of undifferentiated keratinocytes [74]. A differentiated and strictly regulated CXCR3-A and CXCR3-B expression on keratinocytes and fibroblasts can be reasonably hypothesised and contributes to this pathway, but still needs to be proved. The possible roles of chemokines in the different steps of inflammatory processes from the starting tissue injury until the healing phase are summarised in Figure 1.

De novo blood vessel formation

Previous and more recent evidences indicate that ECs express specific receptors, which can account for an important role of chemokines in angiogenesis (Fig. 2A). Receptors for angiogenic chemokines expressed by ECs include CXCR1, CXCR2 and CXCR4 [75]. The first angiogenic chemokine receptor identified so far is CXCR4. *CXCR4*/*CXCL12*-deficient mice die prenatally and exhibit defects in the formation of gastrointestinal tract arteries, as well as defects in vessel development, haematopoiesis and cardiogenesis [1, 2]. The existence of a regulatory loop between VEGF-A and CXCL12/CXCR4 further supports the important role of this chemokine system in the regulation of angiogenesis. Indeed, CXCL12 upregulates VEGF-A production, and VEGF-A upregulates CXCR4 expression, thus generating an amplification circuit, which is critically influenced by hypoxia [76, 77]. Subsequently, the observation of angiogenesis impairment in *CXCR2*-deficient mice has allowed to demonstrate that this receptor mediates the angiogenic activity of CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 and CXCL7.

The understanding of mechanisms responsible for CXC chemokine-mediated angiostatic effects (Fig. 2A) has been more difficult, mainly because CXCL4 and CXCL10 inhibit angiogenesis through both receptor-independent (i.e., competing with heparan sulfate proteoglycans on the cell surface or directly binding to these growth factors) and receptor-dependent mechanisms [78–80]. Recently, however, CXCR3 has been clearly detected in ECs, particularly at level of ECs from small vessels [81]. More importantly, it was found that CXCR3 expression by primary HMVECs was restricted to the S-phase of the cell cycle [81]. Our studies also led to the demonstration that CXCL11, the third known CXCR3-binding chemokine, was able to inhibit EC proliferation [81]. Furthermore, neutralising anti-CXCR3 antibodies blocked the antiproliferative activity induced on ECs by all three known

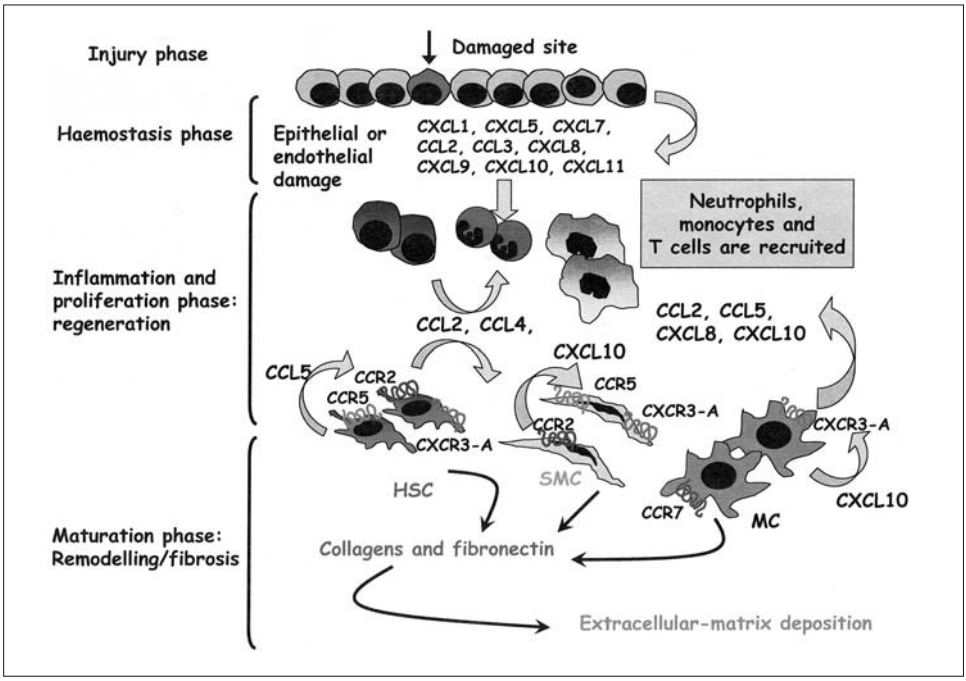

Figure 1

Role of chemokines in the different phases of inflammatory processes

In different tissues, the wound healing response shares many similarities, involving the recruitment of inflammatory cells and the deposition of extracellular matrix, to fill the gap created by the dying cells. Indeed, after tissue damage, chemokines such as CXCL1, CXCL5, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CCL2, CCL3, lead to the recruitment of monocytes/macrophages, T cells and neutrophils. The concurrent presence of inflammation and extracellular matrix deposition is a characteristic of chronic tissue injury, where the persistence of a wound healing response may lead to permanent scarring and end-stage organ failure, such as in the case of glomerulosclerosis in the kidney, cirrhosis of the liver, atherosclerosis, or pulmonary fibrosis. The pivotal role played by vascular pericytes of different tissues in the process of wound healing has been clearly recognised in recent years. These cells become activated in the presence of damage to the specific tissue, proliferate, migrate, and acquire a myofibroblast-like phenotype, resulting in the production of extracellular matrix as part of the healing process. Pericytes responsible for tissue fibrosis may express chemokines such as CCL2, CCL4, CCL5, CXCL8, CXCL10, thus contributing to the pathogenesis of the inflammatory reaction. Furthermore, pericytes can also be targets of the action of chemokines, since they express chemokine receptors, such as CXCR3-A, CCR2, CCR5, CCR7, which, upon activation, elicit biologic actions that favour the wound healing process, including proliferation, migration, and extracellular matrix synthesis.

CXCR3 ligands, thus definitively proving that CXCR3 is the receptor involved in CXC chemokine-mediated angiostatic activity [81]. The role of CXCR3 in mediating the angiostatic activity of CXCL10 has recently been confirmed *in vivo* by blocking the angiostatic effects of CXCL10 in the rat cornea micropocket assay with a neutralising anti-CXCR3 antibody [82].

Some questions, however, still needed to be solved. First, the receptor for CXCL4, the most powerful angiostatic chemokine, remained unknown, despite the fact that this chemokine shares many activities with CXCL10. On the other side, CXCR3-binding chemokines also exhibit powerful chemotactic activity, whereas the CXCL4-mediated chemotactic effect is modest or absent [83]. Finally, the opposite effects exerted by CXCR3 ligands on HMVECs (inhibition of proliferation) and on vascular pericytes (increase of proliferation) [31, 84–86] allow to hypothesise the existence of cell-specific signal transduction pathways or even of distinct CXCR3 receptor variants.

Indeed, a distinct, previously unrecognised receptor, deriving from an alternative splicing of the *CXCR3* gene, was identified, which not only mediates the angiostatic activity of the three already known CXCR3 ligands, but also acts as functional receptor for CXCL4 [71]. By contrast, the known CXCR3, renamed CXCR3-A, mediated the proliferation of vascular pericytes in response to CXCL9, CXCL10 and CXCL11, whereas it bound CXCL4 with very low affinity [71]. Finally, monoclonal antibodies, that were selectively developed against CXCR3-B, reacted with ECs of different human tumour tissues but poorly, or not, with those from their normal counterparts, consistently with the previously described selective effects of both CXCL4 and CXCL10 on actively proliferating ECs [71]. Of note, another form of CXCL4 (CXCL4L1) has recently been isolated from thrombin-stimulated human platelets, which differed from CXCL4 in only three amino acids, and appeared to be more potent in inhibiting chemotaxis of HMVECs toward CXCL8 or bFGF [87]. Notably, a third variant of human CXCR3 (CXCR3-alt) resulting from alternative splicing via post-transcriptional exon skipping has also been identified [88]. However, the functional activity of this variant is not yet known.

Tumour formation

The course in angiogenesis usually correlates with the degree of infiltration by inflammatory leukocytes [59]. The coordination of angiogenesis and inflammation is due to the ability shared by ECs and leukocytes to respond to chemokines [61].

In physiologic processes, such as wound healing, the interplay of CXC chemokines with growth factors, cytokines and adhesion molecules regulates the events leading to angiogenesis. The repair process is initiated immediately after injury of blood vessels through the release of platelets-derived factors as described above. CXCL8 expression by wounded epithelial cells induces massive angiogene-

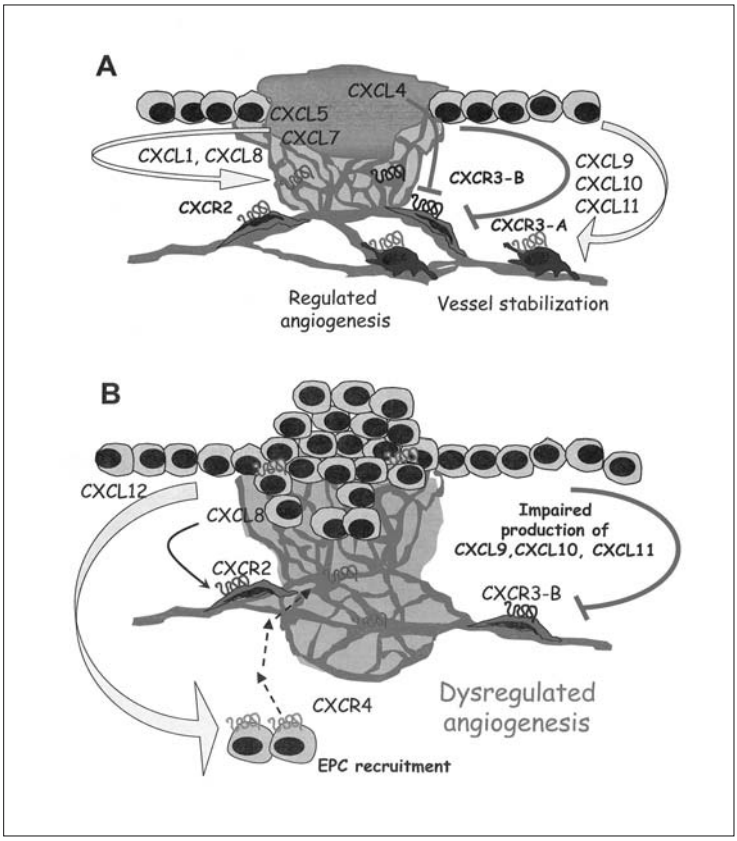

Figure 2
Role of chemokines in physiologic and dysregulated angiogenesis
(A) On wounding or tissue assault, platelets are activated and form a haemostatic plug, in which they release vasoactive mediators that regulate formation of the fibrin clot. CXCL1, CXCL5, CXCL7, derived from activated platelets, initiate the recruitment of neutrophils. Subsequently, CXCL8 expression by wounded epithelial cells induces massive angiogenesis, leading to the formation of new blood vessels that exhibit high CXCR2 expression. Conversely, expression of the angiostatic chemokines CXCL9, CXCL10, and CXCL11 prevents unlimited vessel growth, arresting migration and growth of proliferating endothelial cells, which selectively express CXCR3-B.
(B) An altered balance of CXC chemokines might be crucial in contributing to cancer development during chronic inflammatory processes through different mechanisms. Excessive production of angiogenic chemokines, such as CXCL8, and their receptor CXCR2, can lead to a level of inflammation that potentiates angiogenesis. Poor expression of angiostatic chemokines and of their receptor, CXCR3-B, can lead to a level of inflammation that potentiates angiogenesis or can directly alter the proliferative properties of resident epithelial cells.

sis, leading to the formation of new blood vessels expressing functional CXCR2 [64, 66]. Conversely, expression of the angiostatic chemokines CXCL9 and CXCL10 prevents unlimited vessel growth arresting migration and growth of proliferating ECs expressing CXCR3-B. CXCL10, CXCL9 and CXCL11 also mediate the migration of CXCR3-A-expressing pericytes and their proliferation around nascent vessels, thus determining their stabilisation.

On the other hand, tumours are described as "wounds that never heal" and appear to lack the appropriate balances between positive and negative control signals [89]. One of the main features of tumour blood vessels is their failure to become quiescent, enabling the constant growth of new tumour blood vessels [89]. Consequently, the tumour vasculature develops unique characteristics and becomes quite distinct from existing capillaries. Furthermore, the inappropriate or decreased vessel association with pericytes in tumours might account for both abnormal vessel diameters and sensitivity to VEGF inhibition [89].

Overexpression of angiogenic CXC chemokines favours the "tumour angiogenesis switch" and ultimately leads to tumour progression [89]. Lung colonisation and spontaneous metastasis in nude mice are inhibited by treatment with neutralising antibody against IL-8 [90]. Furthermore, CXCL8 expression in astrocytoma increases during tumour progression, due to reduced microenvironmental oxygen pressure and promotes angiogenesis by binding to CXCR2 [91]. CXCL8 and GRO-α are also induced by Kaposi Sarcoma Herpes Virus (KSHV) infection of endothelial cells and are crucial to the angiogenic phenotype developed by KSHV-infected ECs in cell culture and upon implantation into SCID mice [92]. A few data are available on the role of CXCL12 in angiogenesis progression in tumours. However, CXCL12 can contribute to tumour neovascularisation through vasculogenesis-mediated by EC precursors. Indeed, locally derived CXCL12 augments vasculogenesis and contributes to ischemic neovascularisation *in vivo* by augmenting the recruitment and survival of EC precursors [93]. Conversely, angiostatic chemokines play an important role in fighting tumour development and diffusion. Indeed, overexpression of CXCL4 and CXCL10 blocks tumour progression and can also induce regression of metastasis [94, 95]. The possibility that inadequate expression of CXCR3-B by angiogenic ECs during a chronic inflammatory process might favour the "tumour angiogenesis switch" might also be hypothesised. In 40 patients affect-

Resident epithelial cells undergo neoplastic progression and then, following hypoxia, "turn on" the expression of CXCR4. The production of CXCL12 in sites, such as lymph nodes, bone marrow, liver, and lung, then facilitates their invasion and migration to secondary sites to form a productive metastatic lesion and also potentiates angiogenesis, through its interaction with CXCR4. On the other hand, impaired production of CXCL9, CXCL10 and CXCL11 and/or their receptor CXCR3-A can result in impaired recruitment and activation of inflammatory cells resulting in escape of the tumour from immune surveillance.

ed by non small cell lung cancer (NSCLC), we observed a significant inverse correlation between CXCR3-B mRNA expression and both tumour stage and rate of lymph node invasion (Lazzeri E et al. manuscript in preparation). An inverse correlation between CXCR3-B expression and angiogenesis was only observed among patients with localised tumours and without lymph node invasion, suggesting that the loss of angiogenesis regulation by CXCR3-B might favour NSCLC diffusion. Similar findings were found in patients with renal cell carcinoma (Lazzeri E et al., manuscript in preparation). Collectively, dysregulation of chemokine production and/or interaction of chemokines with their receptor(s) appear to play an important role in the growth of cancer and in the formation of metastases. Figure 2B shows the possible role of different chemokines in the dysregulation of angiogenesis which occurs in neoplastic processes.

Chemokines control of other tissue cells

Many cell types in the brain express chemokines and chemokine receptors even under homeostatic conditions, arguing for a role of these molecules in normal brain processes. It has indeed been shown that CXCL12 and CCR3-binding chemokines reversibly inhibit neuronal progenitor cell (NPC) proliferation in isolated cells, neurospheres, and in hippocampal slice cultures [96]. On the other hand, CX3CL1 has been found to be able to promote survival of NPCs [96].

Cells of the central nervous system

There is also growing evidence for the role of chemokines in the regulation of central nervous system (CNS) diseases. Elevated levels of chemokines have been indeed observed in both experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS), suggesting that these molecules act as regulators of brain inflammation [97, 98]. However, chemokines not only function as key mediators which promote leukocyte infiltration of demyelinating lesions in both EAE and MS, but they also act on microglia and astrocytes by inducing their migration to sites of inflammation, and their proliferation that could represent the basis of pathological conditions such as gliosis. The major receptors on these cells appear to be CXCR1 and CXCR3, but also CCR3 [99].

Osteoclasts

Although much has been learned of the mechanisms by which the migration and differentiation of osteoclasts (OCs) are induced, only recently the essential role of

chemokines in this process has been recognised. CXCL12 stimulates matrix metalloproteinase-9 activity on pre-OCs, thus favouring their recruitment to sites for OC differentiation and bone readsorption [100]. On the other side, CXCL8 has been shown to play a direct effect on OC differentiation and activity by interacting with its specific receptor CXCR1, which appears to be expressed on the surface of these cells [101]. CCL9 and its receptor CCR1 have also been found on OCs, suggesting that this chemokine and its receptor may also play a role in the regulation of bone readsorption [102]. Moreover, high levels of CCL3 have been found in bone marrow samples from patients with multiple myeloma, suggesting that it may be one of the major factors responsible for the increased OC stimulatory activity in patients with this disease [103]. However, a more recent study, based on the use of gene array, showed that of all the mediators screened, CCL15 was the most strongly upregulated in stimulated OC precursors [104]. More importantly, neutralisation of CCL15 resulted in strongly reduced OC formation and reduced resorptive activity, since CCL15 also promoted OC survival and prevented OC apoptosis. These results suggest that OCs can protect themselves from apoptosis through production of CCL15 as an autocrine survival factor [104].

Conclusions

Chemokines are secretory proteins produced by leukocytes and tissue cells either constitutively or after induction, and exert their effects locally in paracrine or autocrine fashion via their binding to heptahelical G-protein coupled receptors. The increase in the secretion of chemokines during inflammation results in the selective recruitment of leukocytes into inflamed tissues such as skin, brain, lung, kidneys and gastrointestinal tract. In these organs many types of cells secrete chemokines, suggesting that, if the appropriate stimulus is given, most cells can secrete chemokines.

Moreover, in organs such as kidney, lung and liver, chemokines may play an important role in the maintenance of tissue homeostasis, in local remodelling processes and may modulate the progression of fibrosis by acting on tissue specific pericytes. Most importantly, chemokines have been found to have a main role in the regulation of angiogenesis and tumour-related immunity, and in promoting organ-specific metastases.

Our knowledge on the roles of chemokines in the pathophysiology of disease are derived from studies utilising animal models of disease and mice with deleted chemokine receptor genes. The main problems in studying the role of chemokines in these models might be represented by the great redundancy shown by the chemokine system (i.e., different chemokines can bind a single chemokine receptor and a single chemokine can bind more than a receptor) and some differences between species in the expression of chemokines and chemokine receptors and in their binding properties. However, there is growing evidence that the neutralisation

of chemokine activity may have a therapeutic value. Indeed, chemokine analogues with antagonist or partial agonist activity proved effective in animal models as inhibitors of inflammatory pathologies. In particular, given the role of chemokines in excessive fibrosis, novel strategies aimed at preventing fibrotic disease will likely need to address the early engagement of inflammatory cells by tissue epithelial and interstitial cells, and possibly modulate the ability of resident tissue cells to generate and/or recognise profibrotic signals supplied by chemokines. Finally, understanding the biology of factors that contribute to cancer tumourigenicity, avoidance of host immunity, metastases and angiogenesis may lead to novel strategies for therapeutic intervention of this devastating disease.

References

1 McGrath KE, Koniski AD, Maltby KM, McGann JK, Palis J (1999) Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. *Developm Biol* 213: 442–456

2 Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S-I, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF1. *Nature* 382: 635–638

3 Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, Kitamura Y, Matsushima K, Yoshida N, Nishikawa S-I et al (1998). The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* 393: 591–594

4 Grone H-J, Cohen CD, Grone E, Schmidt C, Kretzler M, Schlondorff D, Nelson PJ (2002) Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing human kidney. *J Am Soc Nephrol* 13: 957–967

5 Stebler J, Spieler D, Slanchev K, Molyneaux KA, Richter U, Cojocaru V, Tarabykin V, Wylie C, Kessel M, Raz E (2004). Primordial germ cell migration in the chick and mouse embryo: the role of the chemokine SDF-1/CXCL12. *Developm Biol* 272: 351–361

6 Sato Y, Higuchi T, Yoshioka S, Tatsumi K, Fujiwara H, Fujii S (2003) Trophoblasts acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype. *Development* 130: 5519–5532

7 Haringman JJ, Kraan MC, Smeets TJ, Zwinderman KH, Tak PP (2003) Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. *Ann Rheum Dis* 62: 715–721

8 Dogan RN, Karpus WJ (2004) Chemokines and chemokine receptors in autoimmune encephalomyelitis as a model for central nervous system inflammatory disease regulation. *Front Biosci* 9: 1500–1505

9 MacDermott RP, Sanderson IR, Reinecker HC (1998) The central role of chemokines (chemotactic cytokines) in the immunopathogenesis of ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 4: 54–67

10 D'Ambrosio D, Mariani M, Panina-Bordignon P, Sinigaglia F (2001) Chemokines and

their receptors guiding T lymphocyte recruitment in lung inflammation. *Am J Respir Crit Care Med* 164: 1266–1275

11 Agostini C, Meneghin A, Semenzato G (2002) T-lymphocytes and cytokines in sarcoidosis. *Curr Opin Pulm Med* 8: 435–440

12 Bisset LR, Schmid-Grendelmeier P (2005) Chemokines and their receptors in the pathogenesis of allergic asthma: progress and perspective. *Curr Opin Pulm Med* 11: 35–42

13 Boisvert WA (2004) Modulation of atherogenesis by chemokines *Trends Cardiovasc Med* 14: 161–165

14 Wakugawa M, Nakamura K, Akatsuka M, Kim SS, Yamada Y, Kawasaki H, Tamaki K, Furue M (2001) Expression of CC chemokine receptor 3 on human keratinocytes *in vivo* and *in vitro* –upregulation by RANTES. *J Dermatol Sci* 25: 229–235

15 Kulke R, Bornscheuer E, Schluter C, Bartels J, Rowert J, Sticherling M, Christophers E (1998) The CXC receptor 2 is overexpressed in psoriatic epidermis. *J Invest Dermatol* 110: 90–94

16 Satish L, Blair HC, Glading A, Wells A (2005) Interferon-inducible protein 9 (CXCL11)-induced cell motility in keratinocytes requires calcium flux-dependent activation of mucalpain. *Mol Cell Biol* 25: 1922–1941

17 Lundien MC, Mohammed KA, Nasreen N, Tepper RS, Hardwick JA, Sanders KL, Van Horn RD, Antony VB (2002) Induction of MCP-1 expression in airway epithelial cells: role of CCR2 receptor in airway epithelial injury. *J Clin Immunol* 22: 144–152

18 Stellato C, Brummet ME, Plitt JR, Shahabuddin S, Baroody FM, Liu MC, Ponath PD, Beck LA (2001) Expression of the C–C chemokine receptor CCR3 in human airway epithelial cells. *J Immunol* 166: 1457–1461

19 Eddleston J, Christiansen SC, Zuraw BL (2002) Functional expression of the C-X-C chemokine receptor CXCR4 by human bronchial epithelial cells: regulation by proinflammatory mediators. *J Immunol* 169: 6445–6451

20 Dwinell MB, Eckmann L, Leopard JD, Varki NM, Kagnoff MF (1999) Chemokine receptor expression by human intestinal epithelial cells. *Gastroenterology* 117: 359–367

21 Yang CC, Ogawa H, Dwinell MB, McCole DF, Eckmann L, Kagnoff MF (2005) Chemokine receptor CCR6 transduces signals that activate p130Cas and alter cAMP-stimulated ion transport in human intestinal epithelial cells. *Am J Physiol Cell Physiol* 288: C321–C328

22 Vlahakis SR, Villasis-Keever A, Gomez TS, Bren GD, Paya CV (2003) Human immunodeficiency virus-induced apoptosis of human hepatocytes via CXCR4. *J Infect Dis* 188: 1455–1460

23 Efsen E, Grappone C, DeFranco RM, Milani S, Romanelli RG, Bonacchi A, Caligiuri A, Failli P, Annunziato F, Pagliai G et al (2002) Up-regulated expression of fractalkine and its receptor CX3CR1 during liver injury in humans. *J Hepatol* 37: 39–47

24 Patterson BK, Landay A, Andersson J, Brown C, Behbahani H, Jiyamapa D, Burki Z, Stanislawski D, Czerniewski MA, Garcia P (1998) Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. *Am J Pathol* 153: 481–490

25 Huber TB, Reinhardt HC, Exner M, Burger JA, Kerjaschki D, Saleem MA, Pavenstadt H (2002) Expression of functional CCR and CXCR chemokine receptors in podocytes. *J Immunol* 168: 6244–6252
26 Han GD, Koike H, Nakatsue T, Suzuki K, Yoneyama H, Narumi S, Kobayashi N, Mundel P, Shimizu F, Kawachi H (2003) IFN-inducible protein-10 has a differential role in podocyte during Thy 1.1 glomerulonephritis. *J Am Soc Nephrol* 14: 3111–3126
27 Gharaee-Kermani M, Denholm EM, Phan SH (1996) Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. *J Biol Chem* 271: 17779–17784
28 Wang X, Yue TL, Ohlstein EH, Sung CP, Feuerstein GZ (1996) Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation, and inflammatory response. *J Biol Chem* 271: 24286–24293
29 Schecter AD, Rollins BJ, Zhang YJ, Charo IF, Fallon JT, Rossikhina M, Giesen PL, Nemerson Y, Taubman MB (1997) Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. *J Biol Chem* 272: 28568–28573
30 Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, Pinzani M, Laffi G, Montalto P, Gentilini P (1999) Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* 29: 140–148
31 Romagnani P, Beltrame C, Annunziato F, Lasagni L, Luconi M, Galli G, Cosmi L, Maggi E, Salvadori M, Pupilli C et al (1999) Role for interactions between IP-10/Mig and CXCR3 in proliferative glomerulonephritis. *J Am Soc Nephrol* 10: 2518–2525
32 Banas B, Luckow B, Moller M, Klier C, Nelson PJ, Schadde E, Brigl M, Halevy D, Holthofer H, Reinhart B et al (1999) Chemokine and chemokine receptor expression in a novel human mesangial cell line. *J Am Soc Nephrol* 10: 2314–2322
33 Schecter AD, Calderon TM, Berman AB, McManus CM, Fallon JT, Rossikhina M, Zhao W, Christ G, Berman JW, Taubman MB (2000) Human vascular smooth muscle cells possess functional CCR5. *J Biol Chem* 275: 5466–5471
34 Hayes IM, Jordan NJ, Towers S, Smith G, Paterson JR, Earnshaw JJ, Roach AG, Westwick J, Williams RJ (1998) Human vascular smooth muscle cells express receptors for CC chemokines. *Arterioscler Thromb Vasc Biol* 18: 397–403
35 Ikeda U, Ikeda M, Seino Y, Takahashi M, Kasahara T, Kano S, Shimada K (1993) Expression of intercellular adhesion molecule-1 on rat vascular smooth muscle cells by pro-inflammatory cytokines. *Atherosclerosis* 104: 61–68
36 Schecter AD, Rollins BJ, Zhang YJ, Charo IF, Fallon JT, Rossikhina M, Giesen PL, Nemerson Y, Taubman MB (1997) Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. *J Biol Chem* 272: 28568–28573
37 Wang N, Tabas I, Winchester R, Ravalli S, Rabbani LE, Tall A (1996) Interleukin 8 is induced by cholesterol loading of macrophages and expressed by macrophage foam cells in human atheroma. *J Biol Chem* 271: 8837–8842
38 Gao JL, Kuhns DB, Tiffany HL, McDermott D, Li X, Francke U, Murphy PM (1993)

Structure and functional expression of the human macrophage inflammatory protein 1 alpha/RANTES receptor. *J Exp Med* 177: 1421–1427

39 Kitaura M, Nakajima T, Imai T, Harada S, Combadiere C, Tiffany HL, Murphy PM, Yoshie O (1996) Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC chemokine receptor 3. *J Biol Chem* 271: 7725–7730

40 Youn BS, Kim SH, Lyu MS, Kozak CA, Taub DD, Kwon BS (1997) Molecular cloning and characterization of a cDNA, CHEMR1, encoding a chemokine receptor with a homology to the human C-C chemokine receptor, CCR-4. *Blood* 89: 4448–4460

41 Hora K, Satriano JA, Santiago A, Mori T, Stanley ER, Shan Z, Schlöndorff D (1992) Receptors for IgG complexes activate synthesis of monocyte chemoattractant peptide 1 and colony-stimulating factor 1. *Proc Natl Acad Sci USA* 89: 1745–1749

42 Pai R, Ha H, Kirschenbaum MA, Kamanna VS (1996) Role of tumor necrosis factor-on mesangial cell MCP-1 expression and monocyte migration: Mechanisms mediated by signal transduction. *J Am Soc Nephrol* 7: 914–923

43 Largen PJ, Tam FW, Rees AJ, Cattell V (1995). Rat mesangial cells have a selective role in macrophage recruitment and activation. *Exp Nephrol* 3: 34–39

44 Wolf G, Aberle S, Thaiss F, Nelson PJ, Krensky AM, Neilson EG, Stahl RA (1993) TNF alpha induces expression of the chemoattractant cytokine RANTES in cultured mouse mesangial cells. *Kidney Int* 44: 795–804

45 Schwarz M, Radeke HH, Resch K, Uciechowski P (1997) Lymphocyte-derived cytokines induce sequential expression of monocyte- and T cell-specific chemokines in human mesangial cells. *Kidney Int* 52: 1521–1531

46 Brown Z, Strieter RM, Chensue SW, Ceska M, Lindley I, Neild GH, Kunkel SL, Westwick J (1991) Cytokine-activated human mesangial cells generate the neutrophil chemoattractant, interleukin 8. *Kidney Int* 40: 86–90

47 Robson RL, Westwick J, Brown Z (1995) Interleukin-1-induced IL-8 and IL-6 gene expression and production in human mesangial cells is differentially regulated by cAMP. *Kidney Int* 48: 1767–1777

48 Gomez Chiarri M, Hamilton TA, Egido J, Emancipator SN (1993) Expression of IP-10, a lipopolysaccharide- and interferon-gamma-inducible protein, in murine mesangial cells in culture. *Am J Pathol* 142: 433–439

49 Duque N, Gomez Guerrero C, Egido J (1999) Interaction of IgA with Fc alpha receptors of human mesangial cells activates transcription factor nuclear factor- B and induces expression and synthesis of monocyte chemoattractant protein-1, IL-8, and IFN-inducible protein 10. *J Immunol* 159: 3474–3482

50 Bonacchi A, Romagnani P, Romanelli RG, Efsen E, Annunziato F, Lasagni L, Francalanci M, Serio M, Laffi G, Pinzani M et al (2001) Signal transduction by the chemokine receptor CXCR3. *J Biol Chem* 276: 9945–9954

51 Banas B, Wörnle M, Berger T, Nelson PJ, Cohen CD, Kretzler M, Pfirstinger J, Mack M, Lipp M, Gröne HJ et al (2002) Roles of SLC/CCL21 and CCR7 in human kidney

for mesangial proliferation, migration, apoptosis and tissue homeostasis. *J Immunol* 168: 4301–4307

52 Banas B, Wornle M, Merkle M, Gonzalez-Rubio M, Schmid H, Kretzler M, Pietrzyk MC, Fink M, de Lema GP, Schlondorff D (2004) Binding of the chemokine SLC/CCL21 to its receptor CCR7 increases adhesive properties of human mesangial cells. *Kidney Int* 66: 2256–2263

53 Marra F, Valente AJ, Pinzani M, Abboud HE (1993) Cultured human liver fat-storing cells produce monocyte chemotactic protein-1. Regulation by proinflammatory cytokines. *J Clin Invest* 92: 1674–1680

54 Czaja MJ, Geerts A, Xu J, Schmiedeberg P, Ju Y (1994) Monocyte chemoattractant protein 1 (MCP-1) expression occurs in toxic rat liver injury and human liver disease. J Leukoc Biol 55: 120–126

55 Marra F, DeFranco R, Grappone C, Milani S, Pastacaldi S, Pinzani M, Romanelli RG, Laffi G, Gentilizi P (1998) Increased expression of Monocyte Chemotactic Protein-1 during active hepatic fibrogenesis: Correlation with monocyte infiltration. *Am J Pathol* 152: 423–430

56 Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, Pinzani M, Laffi G, Montalto P, Gentilini P (1999) Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* 29:140–148

57 Bonacchi A, Petrai I, Defranco RM, Lazzeri E, Annunziato F, Efsen E, Cosmi L, Romagnani P, Milani S, Failli P et al (2003) The chemokine CCL21 modulates lymphocyte recruitment and fibrosis in chronic hepatitis C. *Gastroenterology* 125: 1060–1076

58 Schwabe RF, Bataller R, Brenner DA (2003) Human hepatic stellate cells express CCR5 and RANTES to induce proliferation and migration. *Am J Physiol Gastrointest Liver Physiol* 285: G949–G958

59 Griffioen AW, Molema G (2000) Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. *Pharmacol Rev* 52: 237–268

60 Spinetti G, Camarda G, Bernardini G, Romano Di Peppe S, Capogrossi MC, Napolitano M (2001) The chemokine CXCL13 (BCA-1) inhibits FGF-2 effects on endothelial cells. *Biochem Biophys Res Commun* 289: 19–24

61 Werner S, Grose R (2003) Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 83: 835–870

62 Gillitzer R, Goebeler M (2001) Chemokines in cutaneous wound healing. *J Leukoc Biol* 69: 513–521

63 Shuman MA, Levine SP (1978) Thrombin generation and secretion of platelet Factor 4 during blood clotting. *J Clin Invest* 61: 1102–1106

64 Engelhardt E, Toksoy A, Goebeler M, Debus S, Brocker EB, Gillitzer R (1998) Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. *Am J Pathol* 153: 1849–1860

65 Kemeny L, Szolnoky G, Kenderessy AS, Gyulai R, Kiss M, Michel G, Nagy K, Ruzicka

T, Dobozy A (1994) Role of interleukin-8 receptor in skin. *Int Arch Allergy Immunol* 104: 317–322

66 Devalaraja RM, Nanney LB, Du J, Qian Q, Yu Y, Devalaraja MN, Richmond A (2000) Delayed wound healing in CXCR2 knockout mice. *J Invest Dermatol* 115: 234–244

67 Dipietro LA, Reintjes MG, Low QE, Levi B, Gamelli RL (2001) Modulation of macrophage recruitment into wounds by monocyte chemoattractant protein-1. *Wound Repair Regen* 9: 28–33

68 Fedyk ER, Jones D, Critchley HO, Phipps RP, Blieden TM, Springer TA (2001) Expression of stromal-derived factor-1 is decreased by IL-1 and TNF in dermal wound healing. *J Immunol* 166: 5749–5755

69 Zlotnik A, Yoshie O (2000) Chemokines: a new classification system and their role in immunity. *Immunity* 12: 121–127

70 Rossi D, Zlotnik A (2000) The biology of chemokines and their receptors. *Ann Rev Immunol* 18: 217–242

71 Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, Sagrinati C, Mazzinghi B, Orlando C, Maggi E et al (2003) An alternatively spliced variant of CXCR3 mediates the IP-10, Mig and I-TAC induced-inhibition of endothelial cell growth and acts as functional receptor for PF-4. *J Exp Med* 197: 1537–1549

72 Iocono JA, Colleran KR, Remick DG, Gillespie BW, Ehrlich HP, Garner WL (2000) Interleukin-8 levels and activity in delayed-healing human thermal wounds. *Wound Repair Regen* 8: 216–225

73 Rennekampff HO, Hansbrough JF, Kiessig V, Dore C, Sticherling M, Schroder JM (2000) Bioactive interleukin-8 is expressed in wounds and enhances wound healing. *J Surg Res* 93: 41–54

74 Shiraha H, Glading A, Gupta K, Wells A (1999) IP-10 inhibits epidermal growth factor-induced motility by decreasing epidermal growth factor receptor-mediated calpain activity. *J Cell Biol* 146: 243–254

75 Salcedo R, Oppenheim JJ (2003) Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. *Microcirculation* 10: 359–370

76 Salcedo R, Wasserman K, Young HA, Grimm MC, Howard OM, Anver MR, Kleinman HK, Murphy WJ, Oppenheim JJ (1999) Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: *in vivo* neovascularization induced by stromal-derived factor-1alpha. *Am J Pathol* 154: 1125–1135

77 Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W (2003) Chemokine receptor CXCR4 downregulated by Von Hippel-Lindau tumor suppressor pVHL. *Nature* 425: 307–311

78 Sulpice E, Bryckaert M, Lacour J, Contreres JO, Tobelem G (2002) Platelet factor 4 inhibits FGF2-induced endothelial cell proliferation via the extracellular signal-regulated kinase pathway but not by the phosphatidylinositol 3-kinase pathway. *Blood* 100: 3087–3094

79 Gentilini G, Kirschbaum NE, Augustine JA, Aster RH, Visentin GP (1999) Inhibition of human umbilical vein endothelial cell proliferation by the CXC chemokine, platelet factor 4 (PF-4), is associated with impaired downregulation of $p21^{Cip1/WAF1}$. *Blood* 93: 25–33

80 Jouan V, Canron X, Alemany M, Caen JP, Quentin G, Plouet J, Bikfalvi A (1999) Inhibition of *in vitro* angiogenesis by platelet factor-4-derived peptides and mechanism of action. *Blood* 94: 984–993

81 Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, Uguccioni M, Galli G, Cosmi L, Maurenzig L et al (2001) Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J Clin Invest* 107: 53–63

82 Strieter RM, Belperio JA, Phillips RJ, Keane MP (2004) CXC chemokines in angiogenesis of cancer. *Semin Cancer Biol* 14: 195–200

83 Zucker MB, Katz IR (1991) Platelet factor 4: production, structure, and physiologic and immunologic action. *Proc Soc Exp Biol Med* 198: 693–702

84 Romagnani P, Lazzeri E, Lasagni L, Mavilia C, Beltrame C, Francalanci M, Rotondi M, Annunziato F, Maurenzig L, Cosmi L (2002) IP-10 and Mig production by glomerular cells in human proliferative glomerulonephritis and regulation by nitric oxide. *J Am Soc Nephrol* 13: 53–64

85 Zhao DX, Hu Y, Miller GG, Luster AD, Mitchell RN, Libby P (2002) Differential expression of the IFN-{gamma}-inducible CXCR3-binding chemokines, IFN-inducible protein 10, monokine induced by IFN, and IFN-inducible T Cell {alpha} chemoattractant in human cardiac allografts: association with cardiac allograft vasculopathy and acute rejection. *J Immunol* 169: 1556–1560

86 Wang X, Yue TL, Ohlstein EH, Sung CP, Feuerstein GZ (1996) Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation, and inflammatory response. *J Biol Chem* 271: 24286–24293

87 Struyf S, Burdick MD, Proost P, Van Damme J, Strieter RM (2004) Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor 4/CXCL4 and potent inhibitor of angiogenesis. *Circulation Res* 95: 855–857

88 Ehlert JE, Addison CA, Burdick MD, Kunkel SL, Strieter RM (2004) Identification and partial characterization of a variant of human CXCR3 generated by posttranscriptional exon skipping. *J Immunol* 173: 6234–6240

89 Bergers G, Benjamin LE (2003) Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3: 401–410

90 Rofstad EK, Halsor ER (2000) Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts. *Cancer Res* 60: 4932–4938

91 Desbaillets I, Diserens AC, Tribolet N, Hamou NF, Van Meir EG (1997) Upregulation of interleukin 8 by oxygen-deprived cells in glioblastoma suggests a role in leukocyte activation, chemotaxis, and angiogenesis. *J Exp Med* 186: 1201–1212

92 Lane BR, Liu J, Bock PJ, Schols D, Coffey MJ, Strieter RM, Polverini PJ, Markovitz DM (2002) Interleukin-8 and growth-regulated oncogene α mediate angiogenesis in Kaposi's sarcoma. *J Virol* 76: 11570–11583
93 Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, Bosch-Marce M, Masuda H, Losordo DW, Isner JM et al (2003) Stromal cell-derived factor-1 effects on *ex vivo* expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 107: 1322–1328
94 Tanaka T, Manome Y, Wen P, Kufe DW, Fine HA (1997) Viral vector-mediated transduction of a modified platelet factor 4 cDNA inhibits angiogenesis and tumor growth. *Nat Med* 3: 437–442
95 Homey B, Muller A, Zlotnik A (2002) Chemokines: agents for the immunotherapy of cancer? *Nat Rev Immunol* 2: 175–184
96 Krathwohl MD, Kaiser JL (2004) Chemokines promote quiescence and survival of human neural progenitors. *Stem Cells* 22: 109–118
97 Glabinski AR, Ransohoff RM (1999) Chemokines and chemokine receptors in CNS pathology. *J Neurovirol* 5: 3–12
98 Zhang L, He T, Talal A, Wang G, Framkel SS, Ho DD (2000) Chemokines and chemokine receptors in the pathogenesis of multiple sclerosis. *Mult Scler* 6: 3–13
99 Flynn G, Maru S, Loughlin J, Romero JA, Male D (2003) Regulation of chemokine receptor expression in human microglia and astrocytes. *J Neuroimmunol* 136: 84–93
100 Yu X, Collin-Osdoby P, Osdopy P (2003) SDF-1 increases recruitment of osteoclast precursors by upregulation of matrix metalloproteinase-9 activity. *Connect Tissue Res* 44 suppl 1: 79–84
101 Bendre MS, Montague DC, Peery T, Akel NS, Gaddy, D, Suva LJ (2003) Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. *Bone* 33: 28–37
102 Lean JM, Murphy C, Fuller K, Chambers TJ (2002) CCL9/MIP-1γ and its receptor CCR1 are the major chemokine ligand/receptor species expressed by osteoclasts. *J Cell Biochem* 87: 386–393
103 Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, Alsina M (2000) Macrophage inflammatory protein 1α is a potential osteoclast stimulatory factor in multiple myeloma. *Blood* 15: 671–675
104 Okamatsu Y, Kim D, Battaglino R, Sasaki H, Spate U, Stashenko P (2004) MIP-1γ promotes receptor activator of NF-κB ligand-induced osteoclast formation and survival. *J Immunol* 173: 2084–2090