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Chemokines as mediators of neovascularization

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Abstract

Chemokines are a superfamily of homologous heparin-binding proteins, first described for their role in recruiting leukocytes to sites of inflammation. Chemokines have since been recognized as key factors mediating both physiologic and pathologic neovascularization in such diverse clinical settings as malignancy, wound repair, chronic fibroproliferative disorders, myocardial ischemia and atherosclerosis. Members of the CXC chemokine family, structurally defined as containing the ELR aminoacid motif, are potent inducers of angiogenesis, whereas another subset of the CXC chemokines inhibit angiogenesis. In addition, CCL2, a CC chemokine ligand, has been implicated in arteriogenesis. In this manuscript, we review the current literature on the role of chemokines as mediators of neovascularization.

Introduction

Neovascularization is a general term that incorporates three forms of new blood vessel growth: vasculogenesis, angiogenesis, and arteriogenesis [1]. *Vasculogenesis* is defined as the *de novo* formation of a capillary plexus by endothelial progenitor cells. During embryogenesis, vasculogenesis begins with formation of mesenchymal angioblasts into vascular structures [2]. In the postnatal period, vasculogenesis may be mediated by bone marrow-derived endothelial progenitors [3, 4], but the contribution of vasculogenesis to neovascularization in the adult remains controversial [5]. *Angiogenesis* is defined as the formation of new capillary networks from pre-existing capillaries and is the best understood form of neovascularization. Angiogenesis may proceed by "sprouting" or "intussusception" (the internal division of the preexisting capillary plexus [6, 7]), resulting in the formation of new thin-walled endothelium-lined structures. Both forms of angiogenesis are triggered by tissue hypoxia [8, 9] and are followed by increased expression of hypoxia-inducible factor-1α (HIF-1α) protein, a nuclear transcription factor, which is the primary molecular event stimulating angiogenesis. *Arteriogenesis* is the formation of arteries, which are defined as blood vessels with 3 distinct wall layers and vasomotor properties. The mechanisms leading to arteriogenesis are incompletely defined, and may involve remodeling and enlargement of pre-existing vessels [10] or budding of new vessels from post-capillary venules [11]. Both, however, are mediated by an increase in shear stress. The newly formed arteries, clinically described as collaterals, develop to bypass severe arterial stenoses to connect the proximal (high-pressure region) to the distal (low-pressure region) arterial system [12].

Although the initial triggers for vasculogenesis, angiogenesis and arteriogenesis differ, they are all affected by a variety of inflammatory cells and mediators that, depending on the

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particular physiologic or pathologic setting, modify the neovascularization process [1]. This review will focus on the processes of angiogenesis and arteriogenesis, and will highlight the critical role that chemokines play in these important biologic processes.

Chemokines are a superfamily of homologous 8-10 kDa heparin-binding cytokine molecules that were described for their role in mediating leukocyte recruitment to sites of inflammation. Chemokine ligands and receptors are also recognized as critical mediators of neovascularization in diverse physiologic and pathologic settings and are involved in the pathogenesis of diverse disease settings including chronic inflammation, fibroproliferative disorders, malignancy, wound repair, and more recently, atherosclerosis [13, 14]. The approximately 50 human chemokines are grouped into 4 families on the basis of conserved cysteine residues near their amino terminus, and are designated CC, CXC, C, and CX_3C sub-families. In the CC chemokine family, the first two cysteine residues are adjacent, whereas the defining feature of the CXC chemokine family is that the first 2 cysteine residues are separated by a non-conserved amino acid, thus constituting the Cys-X-Cys or ‘CXC’ motif. The CXC chemokine ligands are further subdivided on the basis of the presence or absence of another 3 amino acid sequence, glutamic acid-leucine-arginine (the ‘ELR’ motif), immediately proximal to the CXC sequence. The ELR-positive CXC chemokines, which include IL-8/CXCL8, are potent neutrophil chemoattractants. Among the ELR-negative CXC chemokine ligands, CXCL9, CXCL10 and CXCL11 (previously designated MIG, IP-10 and I-TAC, respectively), are potently induced by both type 1 and type 2 interferons (IFN-α/β and IFN-γ) and attract mononuclear leukocytes, including activated Th-1 CD4 T cells, NK cells, and monocytes and myeloid dendritic cells to sites of inflammation. With regards to their role in neovascularization, the CC chemokine ligand CCL2 has also been shown to be a potent promoter of arteriogenesis. Among the CXC chemokines, the ELR-containing CXC chemokines are potent *promoters* of angiogenesis, whereas the interferon-inducible subset of the ELR-negative CXC chemokines are potent *inhibitors* of angiogenesis [15, 16] (Table).

Chemokines that promote neovascularization

All ELR-positive CXC chemokines are potent promoters of angiogenesis (Table). In the mouse, all ELR-positive CXC chemokine ligands signal via CXCR2, whereas the human ELR-positive CXC chemokines signal through 2 receptors, CXCR1 and CXCR2. Several observations support the notion that human CXCR2 is the primary receptor for chemokine-mediated angiogenesis: (1) all human ELR-positive CXC chemokines mediate angiogenesis and can bind CXCR2, whereas only two of these ligands, CXCL8 and CXCL6, have the ability to bind to CXCR1; (2) while both CXCR1 and CXCR2 are both expressed by endothelial cells, only the expression of CXCR2 is required for endothelial cell chemotaxis [17, 18]; and (3) when the function of CXCR2 is blocked, the response of endothelial cells to CXCL8 is abrogated [19].

CXCR2 activation by the ELR-positive CXC chemokines can lead either to receptor internalization, receptor degradation, or recycling of the receptor to the cell membrane. CXCR2 internalization is critical to the generation of a chemotactic response: a mutation of this receptor which impairs internalization has been shown to markedly reduce chemotaxis [20]. In this context, the local concentration of ligands dictates the fate of CXCR2: in the setting of low concentrations of ligand, internalized CXCR2 is targeted for recycling and returns to the cell surface whereas high concentrations of ELR-positive CXC chemokines result in targeting of internalized CXCR2 to endosomes for recycling or lysosomes for receptor proteolysis [20].

CXCR2 plays an integral role in mediating ELR-positive CXC chemokine angiogenesis in the cornea micropocket model in CXCR2+/+ and CXCR2 knockout mice [17]: the ELR-positive CXC chemokine-mediated angiogenesis was inhibited in CXCR2 knockout mice and in the presence of CXCR2 neutralizing antibodies. In addition, recent reports have suggested that

CXCR2 and ELR+ CXC chemokine ligands can mediate homing of circulating endothelial progenitor cells to sites of arterial injury [21, 22]. These studies provide evidence that the CXCR2 receptor is important to ELR-positive CXC chemokine-mediated angiogenesis.

Another receptor shown to modulate the angiogenic effects of the ELR-positive CXC chemokines is the Duffy antigen receptor for chemokines (DARC). This receptor is a chemokine receptor that binds chemokines in the absence of detectable signal transduction events [23, 24]. It acts as a decoy receptor that inhibits angiogenesis by sequestering ELR-positive CXC chemokines CXCL1, CXCL5 and CXCL8. Transgenic expression of DARC on mouse endothelial cells resulted in decreased angiogenic response of the animals to ELR-positive CXC chemokines in vivo [25]. In a mouse model of prostate cancer, animals on a DARC-deficient background developed larger and more aggressive tumors with greater tumor-associated neovascularization and increased intra-tumor levels of angiogenic ELR-positive CXC chemokines [26]. Similarly, in a human non-small cell lung cancer tumor cell line, over-expression of DARC resulted in binding of angiogenic ELR-positive CXC chemokines by the tumor cells and a marked decrease in tumor-mediated angiogenesis and metastases [24].

In addition to the CXC chemokine family, 3 members of the CC chemokine family, CCL2, CCL11 and CCL16, have also been implicated in neovascularization. CCL11 signals via the receptor CCR3 and mediates chemotaxis of human endothelial cells and promotes neovascularization in several models of angiogenesis, including chick chorioallantoic membrane neovascularization and Matrigel plug assays [27]. CCL16 is primarily expressed in the liver, suggesting that it may play a role in the liver's vascular development and in angiogenesis associated with hepatic diseases [28]. CCL16 has been shown to induce the migration of endothelial cells, promote endothelial differentiation into capillary-like structures, and to mediate angiogenesis in chick chorioallantoic membrane by activating CCR1 [28].

CCL2 is the best-described CC chemokine mediator of neovascularization. Endothelial cells express CCR2, the receptor for CCL2, and display demonstrate chemotaxis and tube formation in response to CCL2 *in vitro*[29-31]. CCL2-mediated neovascularization has also been demonstrated in *in vivo* models including the Matrigel plug, chick chorioallantoic membrane, sponge and corneal implantation models [32-34]. CCL2-mediated angiogenesis appears to be dependent on membrane type 1-matrix metalloproteinase (MT1-MMP): the absence or blockade of MT1-MMP activity resulted in decreased *in vivo* and *in vitro* angiogenesis induced by CCL2 [30]. CCL2 also significantly induced MT1-MMP surface expression, clustering, activity, and function in human endothelial cells. The angiogenic effect of CCL2 appears to be independent of its effects on leukocyte chemotaxis and is mediated via direct effects on the vascular endothelium [34] and may also mediate homing of endothelial progenitor cells to sites of vascular injury [35]. Lastly, *in vivo* CCL2-induced angiogenesis has been associated with both the induction of vascular endothelial growth factor (VEGF)-A gene expression [36], and the transcription factor, MCP-1 induced protein [37].

CXC Chemokines that inhibit neovascularization

Several ELR-negative CXC chemokines have been implicated as inhibitors of angiogenesis (Table). Among these, human CXCL4, CXCL9, CXCL10, and CXCL11 all mediate angiostasis via the receptor CXCR3. The CXCR3 receptor, which was originally identified on murine endothelial cells [38], has been shown to mediate angiostatic activity [39]. This receptor is unique in that it exists in three different splice variants, CXCR3A, CXCR3B, and CXCR3-alt, all generated by alternative splicing of mRNA of a single gene product. The expression of CXCR3A is strongly induced by interleukin (IL)-2, and it is primarily responsible for recruitment of leukocytes, most notably of Th1 lymphocytes [40-46]. Conversely, CXCR3B is the main angiostatic variant of CXCR3 and is expressed on endothelial cells [39, 47, 48]. The final variant, CXCR3-alt, is the most recently described and has been shown to have greater affinity for CXCL11 as compared to CXCL9 or CXCL10, but its role in angiogenesis is yet to be determined [49]. CXCR3B is the main angiostatic receptor for CXCL4, CXCL9, CXCL10, and CXCL11 [50, 51]. The angiostatic chemokines, CXCL9, CXCL10, and CXCL11 are strongly induced by both type I and type II interferons (IFN-α/β and IFN-γ, respectively). These interferon-inducible ELR-negative CXC chemokines are potent inhibitors of angiogenesis in response to the angiogenic ELR-positive CXC chemokines, as well as to VEGF and basic fibroblast growth factor (bFGF). CXCL4, the first angiostatic CXC chemokine described [52], is a potent inhibitor of endothelial cell chemotaxis and proliferation, and has been shown to inhibit the angiogenic effect of VEGF and bFGF [53].

A unique feature of the CXCR3 ligands is that they mediate two distinct effects, namely inhibition of angiogenesis and promoting Th1-type cell mediated immunity via recruitment of CXCR3-expressing T and NK cells [41, 43, 45, 46, 54]. The local production of IFN-γ at the site of inflammation induces a self-perpetuating cycle, promoting further expression of CXCL9, CXCL10, and CXCL11 and recruiting CXCR3-expressing cells that act as a further source of IFN-γ. These combined effects, which we have described as “immunoangiostasis”, can benefit the host in the context of anti-tumor immunity [55, 56]. For example, in the context of renal cell carcinoma, the effectiveness of systemic IL-2 therapy was shown to be dependent on the receptor CXCR3; the therapy resulted in the up-regulation of CXCR3 on peripheral blood mononuclear cells, but the down-regulation of its ligands within the tumor. These anti-tumor effects of systemic IL-2 were substantially amplified when systemic administration of IL-2 was combined with over-expression of CXCL9 in the tumor, thereby augmenting the homing of IFN-γ producing leukocytes to the tumor microenvironment, inhibiting tumor-associated angiostasis, and enhancing immune responses against tumor antigens [55]. Importantly, immunoangiostasis is operative not only in the context of IL-2-mediated effects in renal cell carcinoma, since a similar mechanism has been noted in IL-12-mediated regression of a mouse model of renal cell carcinoma [57]. Similar findings have been shown in non-small cell lung carcinoma [58, 59]; in addition to a reduction in angiogenesis, intratumoral injection of a recombinant CC chemokine, CCL21, induced tumor regression in immunocompetent mice, but not immunosuppressed mice suggesting that T cell immunity was required for the anti-tumor effect of CCL21. Moreover, this was associated with intra-tumor generation of IFN-γ, CXCL9 and CXCL10, and depletion studies demonstrated that CXCL9, CXCL10, and IFN-γ each attenuated the anti-tumor effects of CCL21 [58].

In addition to binding CXCR3, both CXCL4 and CXCL10 ligands also bind to extra-cellular glycosaminoglycans. To determine whether the angiostatic properties of these ligands were mediated via this mechanism studies were performed using CXCL4 and CXCL10 variants with mutated binding sites for CXCR3 or glycosaminoglycans. The angiostatic activity CXCL4 was retained in cells that lacked surface heparin sulfate, and CXCL4 mutants that lacked heparin-affinity are capable of inhibiting angiogenesis [60-62], indicating that interaction with cell surface glycosaminoglycans is not essential for these effects. Similarly, when CXCL10 variants with mutated binding sites for CXCR3 or glycosaminoglycans were transfected into a human melanoma cell line, wild-type CXCL10 and CXCL10 mutants with partial or complete loss of glycosaminoglycans binding promoted significant reduction in tumor growth compared to control vector-transfected tumor cells, whereas tranfectants expressing mutants with loss of the CXCR3 binding domain did not inhibit tumor growth [63]. While these studies demonstrated that the angiostatic effects of CXCR3 ligands are CXCR3-dependent, these ligands can also exert their angiostatic effects by CXCR3-independent mechanisms via interfering with the angiogenic effects of CXCL8, VEGF and bFGF [62, 64, 65]. Heterodimerization with CXCL4 prevents the homodimerization of bFGF that is necessary for receptor binding [62, 66]. Moreover, CXCL4 restricts $VEGF_{165}$ binding to its receptors on endothelial cells by a similar mechanism

[67]. CXCL4 does not, however, appear to bind non-heparin-binding angiogenic peptides: for example, it does not bind to $VEGF_{121}$ or its receptor [67-69].

The ligand-receptor relationship of CXCL4 is further complicated by the existence of its non-allelic variant, CXCL4L1 (previously designated $PF\text{-}4_{var}$ and SCYB4V1). CXCL4L1 differs from CXCL4 in 3 amino acids in the heparin-binding domain near the carboxy terminus [70]. CXCL4L1 protein has been isolated from the α-granules of thrombin-activated human platelets [71]. CXCL4L1 was >30-fold more potent than CXCL4 in inhibiting human microvascular endothelial cell chemotaxis induced by bFGF and CXCL8, and was also more potent in inhibiting in vitro wound-healing assay and bFGF- and CXCL8-induced angiogenesis in the rat corneal micropocket model [71, 72]. CXCL4L1 was also more efficient than CXCL4 in inhibiting tumor-associated angiogenesis in B16 melanoma and A549 lung adenocarcinoma in immunocompromised mice [72].

The CXC chemokine ligand CXCL12 and its receptor, CXCR4, are critical to homing of progenitor cells in diverse biological settings [73]. The precise role of this ligand-receptor pair in angiogenesis is not yet fully established. In the context of cancer biology, CXCR4 is expressed by many tumor lines and primary cancer cells, but its ligand, CXCL12 is not expressed within the cancer microenvironment [73-75]. In contrast to studies of depletion of the ELR+ CXC chemokines and CXCR2, which show a parallel reduction in angiogenesis, tumor size and metastases, depletion of CXCL12 or CXCR4 does not affect tumor size or extent of primary tumor-associated angiogenesis [63, 76, 77]. However, depletion of CXCL12 or CXCR4 was associated with decreased metastases in animal models of breast and lung cancer [74, 75], suggesting that the CXCL12-CXCR4 ligand-receptor pair regulates metastases independent of angiogenesis. On the other hand, CXCL12 is expressed in ischaemic tissues under the control of HIF-1α [78]. In addition, in models of wound healing and several models of tissue hypoxia, CXCL12 mediate homing of endothelial progenitor cells to blood vessel walls in the ischaemic tissue [78-82]. Interestingly, tissue expression of CXCL12 is associated with that of VEGF [83] and can also be induced by transgenic over-expression of VEGF, supporting the notion of cross-talk between chemokine- and cytokine-mediated angiogenesis [84].

Chemokines and neovascularization in human disease

Angiogenic and angiostatic chemokines have been implicated in the neovascularization process that occurs in diverse human diseases, including malignancy, chronic inflammatory and fibroproliferative disorders, wound repair, myocardial ischemia, heart failure and atherosclerosis.

Malignancy

Angiogenesis is essential for the development and progression of tumors; the increased metabolic demand brought on by rapid growth of neoplastic tissue requires commensurate increases in blood supply in order to maintain tissue integrity. When the increased metabolic demands outpace the pre-existing blood supply, a wide range of molecular signals within the microenvironment of the tumor result in the process of angiogenesis. Much of the tumor angiogenesis research has focused on the effects of the VEGF family [85]. However, CXC chemokine-mediated angiogenesis has been shown to play a critical role in growth of many malignancies including lung, pancreatic, ovarian, prostate, melanoma, brain, and renal cell [86-92].

The ELR-positive CXC chemokines CXCL5 and CXCL8 have been shown to play an important role in human non-small cell lung cancer (NSCLC). In a SCID mouse model, human NSCLC tumor-derived CXCL8 levels were directly related to the extent of angiogenesis; when CXCL8 was depleted, however, there was a significant reduction in tumor size, tumor-induced angiogenesis, and metastases [76]. It has also been shown that NSCLC cell lines that constitutively express higher levels of CXCL8 display greater virulence and angiogenic activity in mice [77, 93].

In addition to CXCL8, CXCL5 has been shown play an important role in angiogenesis, and is highly correlated with NSCLC-associated angiogenesis: there is a direct relationship between tissue levels of CXCL5 found in surgical specimens and the extent of capillary density consistent with tumor angiogenesis [94]. Moreover, the expression of ELR-positive CXC chemokines in human NSCLC specimens correlate with clinical outcomes, including mortality [94, 95]. Interestingly, while a significant correlation exists between CXCL5 and tumor-derived angiogenesis, tumor growth, and metastases, CXCL5 depletion does not completely inhibit tumor growth [96]. This is thought to be due to functional redundancy between angiogenic ligands, and has been described in other disease settings [97].

In murine models of NSCLC, when the angiogenic CXC chemokines are neutralized angiogenic activity is decreased, and is followed by a reduction in tumor growth and metastases [23, 76, 96]. In a syngeneic tumor model of lung cancer, CXCR2 knockout mice had reduced tumor growth, increased tumor-associated necrosis, and decreased tumor-associated angiogenesis and metastases compared to wildtype mice [23]. In a different murine model, lung adenocarcinomas in mice with somatic activation of the oncogene KRAS were found to produce ELR-positive CXC chemokines, and again, neutralization of the CXCR2 receptor inhibited tumor development and apoptosis within the tumor [98].

The ELR-positive CXC chemokines have also been studied in human gastrointestinal cancers including pancreatic and colorectal malignancies. Human pancreatic cancer cell lines secrete the ELR-positive angiogenic CXC chemokines CXCL1 and CXCL8 [99], but their expression differs across the different cell lines [97]. When the different cancer cell lines were compared using the corneal micropocket model, tumor-induced angiogenesis was inhibited by blocking the receptor, CXCR2 in one cancer cell line, but not another; again supporting the concept of redundancy of angiogenic ligands, even within specific cancers. In colorectal cancer, in vivo tumor growth is also induced by increased expression of CXCL1 [100].

Human ovarian and prostate cancers are highly dependent on successful angiogenesis for growth and metastatic potential. In one study of human ovarian cancer cell lines, in vitro expression of CXCL8 correlated with increased tumor neovascularization. Importantly, when the tumors were implanted into the peritoneum of immunocompromised mice, the mice had increased mortality rates [101]. In this same study, the expression of VEGF correlated with ascites production, however, it was not associated with either the extent of angiogenesis or with mortality rates [101]. Interestingly, in a separate study, the angiogenic potential of ascites fluid from patients with ovarian cancer was directly correlated with CXCL8 levels [102].

In human prostate cancer, tumorigenesis and metastases are dependent on angiogenesis [103, 104]. Serum CXCL8 levels are markedly elevated in patients with prostate cancer, and these levels correlated with stage of the disease independent of prostate specific antigen levels [104, 105]. In a SCID mouse model of human prostate cancer, different prostate cancer cell lines were found to use different ELR-positive CXC chemokine ligands: depletion of CXCL1 but not CXCL8 inhibited tumor-related angiogenesis in some cell lines, whereas the depletion of CXCL8 but not CXCL1 inhibited angiogenesis in other lines [106].

In patients with malignant melanoma, the angiogenic ELR-positive CXC chemokines, CXCL1, CXCL2 and CXCL3 are highly expressed [107]. Sustained transgenic expression of CXCL1, CXCL2, and CXCL3 in immortalized murine melanocytes transformed their phenotype into one with the ability to form highly vascular tumors in immunocompetent mice [107, 108].

Furthermore, in these same studies, depletion of CXCL1, CXCL2 or CXCL3 in vivo resulted in marked attenuation of tumor-associated angiogenesis and inhibition of tumor growth.

Glioblastoma multiforme tumors are also associated with marked angiogenesis [109, 110]. While the mechanisms responsible with their increased growth and marked angiogenesis remain to be fully defined, a tumor suppressor gene appears to be important and is associated with the expression of angiogenic ELR-positive CXC chemokines in this disease. In one study, a candidate tumor suppressor gene was found to be down-regulated in human glioblastoma specimens compared with normal brain tissue. When implanted into immunocompromised mice, the specimens with the lowest expression of the tumor suppressor gene had the largest growth and degree of angiogenesis [110]. The mechanism for this increased tumorigenicity was found to be CXCL8-dependent; inhibition of CXCL8 in vivo markedly reduced their tumor growth and tumor-associated angiogenesis.

Fibroproliferative disorders

The metabolic demands of proliferating tissue are higher than normal tissue and require an increased blood supply. Angiogenesis, therefore, plays a major role in the pathophysiology of such complex biologic processes as wound repair, chronic inflammatory and fibroproliferative diseases such as rheumatoid arthritis, psoriasis, idiopathic pulmonary fibrosis, bronchiolitis obliterans syndrome, acute respiratory distress syndrome, and atherosclerosis.

The ELR-positive CXC chemokines and their receptor, CXCR2 are important mediators of wound repair [111]. CXCL1 and CXCR2 are expressed during wound healing by keratinocytes and endothelial cells in areas where epithelialization and neovascularization occur [112]. In full-thickness excisional wounds, CXCR2 knockout mice demonstrated delayed healing; which was directly associated with impaired angiogenesis [113]. Angiogenesis is critical to neovascularization of rheumatoid arthritic synovial tissue. In a model using whole human synovial tissue from patients with rheumatoid arthritis, the angiogenic ELR-positive CXC chemokines, CXCL8 and CXCL5 were potent mediators of the angiogenesis in the inflamed synovium compared to normal tissue [114]. Psoriasis, a common inherited skin disease, is characterized by hyperproliferation of epidermal keratinocytes and excessive dermal angiogenesis. In a study of human psoriasis, media conditioned by keratinocytes from psoriatic patients induced a vigorous angiogenic response in the rat corneal micropocket model, and keratinocytes from psoriatic skin exhibited a 10- to 20-fold increase in CXCL8 production [115].

The role of chemokine mediated angiogenesis has been documented in several fibroproliferative lung diseases, including idiopathic pulmonary fibrosis (IPF), allograft bronchiolitis obliterans syndrome (BOS) and acute respiratory distress syndrome (ARDS). IPF is a chronic fibroproliferative lung disease characterized by progressive and disorganized tissue repair. Neovascularization was first recognized in the IPF lung in postmortem studies, and was described as extensive anastomoses between pulmonary and bronchial circulations [116], and was subsequently demonstrated in animal models of bleomycin-induced pulmonary fibrosis [117]. Lung tissue and bronchoalveolar lavage fluid obtained from patients with IPF is strongly angiogenic secondary to over-expression of CXCL8, as compared to CXCL10 in the lung [118].

In a mouse model of bleomycin-induced pulmonary fibrosis, the expression and biological activity of chemokines have been studied [119, 120]. In this model, the angiogenic ELR-positive CXC chemokine, CXCL2/3, was associated with increased pulmonary fibrosis and angiogenesis, while the angiostatic ELR negative CXC chemokine, CXCL10, had the opposite effect. Moreover, depletion of endogenous CXCL2/3, or administration of exogenous CXCL10, resulted in marked attenuation of lung fibrosis and a parallel reduction in angiogenesis. Finally, administration of exogenous CXCL11 in this model resulted in reduced lung fibrosis, as measured by lung collagen deposition, and this effect was abrogated with concomitant blockade of CXCR3 [50]. Bronchiolitis obliterans syndrome is a chronic fibroproliferative disorder of the lung and is the most common cause of death in lung transplant recipients [121]. Human lung samples from patients with BOS demonstrate neovascularization, and lung tissue samples and bronchoalveolar lavage fluid have elevated levels of ELR-positive CXC chemokines. In the corneal micropocket model, there is an increase in angiogenesis, and it is inhibited by neutralizing the receptor, CXCR2 [122]. This CXCR2-dependent mechanism has been confirmed in a mouse model of heterotropic tracheal allograft transplantation [122]. Acute respiratory distress syndrome (ARDS) is a severe manifestation of acute lung injury that quickly progresses to a fibroproliferative phase. Compared to ventilated patients without ARDS, ventilated patients with ARDS have elevated levels of angiogenic chemokines and reduced levels of angiostatic chemokines in bronchoalveolar lavage fluid samples [123].

Ischemic heart disease

Angiogenesis has also been implicated in the progression and instability of atherosclerotic plaques [124-128]. The angiogenic ELR-positive CXC chemokine, CXCL8, has been shown to be over-expressed in human coronary artery plaque atherectomy specimens, as compared to control samples from internal mammary arteries without atherosclerosis [129]. In this study, the CXCL8 co-localized with Factor VIII-related antigen expression on endothelial cells in the atherectomy specimens, and was the major mediator of plaque angiogenic activity in the rat cornea micropocket assay.

While a large number of chemokines are induced in the ischemic myocardium [130, 131], their specific contribution to angiogenesis has not been clearly established. The CXC chemokines CXCL8, CXCL10 and the CC chemokine, CCL2, are reproducibly upregulated in various animal models of myocardial ischemia. The ELR-negative CXC chemokine, CXCL10, is induced in canine [132] and murine [133] myocardial infarction models. It has been postulated that the upregulation of CXCL10 in the infarcted myocardium prevents early angiogenesis and granulation tissue formation until the wound is debrided and a fibrin-based matrix, necessary to support in-growth of tissue, is formed [134].

In a murine model of cardiomyopathy, interstitial fibrosis was preceded by a marked induction of the CC chemokine, CCL2 [135]. The wild type mice exhibited macrophage infiltration in the infarcted myocardium within days, and marked interstitial fibrosis within one week, which was accompanied by ventricular dysfunction. The CCL2 knockout mice, however, had markedly less interstitial fibrosis, less macrophage infiltration, and less ventricular dysfunction. In a different closed-chest model of reperfused murine myocardial infarction, CCL2 knockout mice had decreased and delayed macrophage infiltration into the infarcted tissue, and delayed replacement of myocytes with granulation tissue when compared to wild type mice [136]. The absence of CCL2 resulted in attenuation of post-infarction left ventricular remodeling, a prolonged inflammatory phase, and delayed replacement of injured cardiomyocytes with granulation tissue. Interestingly, while CCL2 deficiency diminished myofibroblast accumulation, it did not significantly affect angiogenesis of the infarcted myocardium. Lastly, in a porcine microembolization model, CCL2 was upregulated and associated with a strong angiogenic response when compared to normal myocardium [137]. CCL2 has also been implicated in ischemia-induced arteriogenesis in a murine hind-limb ischemia model [138]. CCR2 knockout mice had reduced levels of arteriogenesis, and femoral artery occlusion lead to loss of structure and function. Moreover, local infusion of CCL2 into the proximal stump of the occluded femoral artery of rabbits has been shown to markedly increase the rate of arteriogenesis [139].

Conclusion

Chemokines were originally described for their role in recruitment of leukocytes, but have also been shown to play an integral biologic role in neovascularization in diverse human diseases, including cancers, fibroproliferative disorders, and ischemic heart disease. Chemokines may represent novel disease markers or therapeutic targets in these disorders.

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Table

Human chemokine ligands and receptors involved in angiogenesis and arteriogenesis. Modified from references [15] and [16].

Systematic nomenclature	Old nomenclature	Receptor
Angiogenic/arteriogenic		
CXCL1	Gro-α	CXCR2
CXCL2	Gro-β	CXCR2
CXCL3	Gro-γ	CXCR2
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR2
CCL2	MCP-1	CCR2
CCL11	Eotaxin	CCR3
CCL16	HCC-4/LEC	CCR1
Angiostatic		
CXCL4, CXCL4L1	PF-4, PF-4_{var}	CXCR3B[*]
CXCL9	Mig	CXCR3B
CXCL10	IP-10	CXCR3B
CXCL11	I-TAC	CXCR3B
CXCL12	SDF-1	CXCR4

[*] glycosaminoglycan binding may be involved, see text for details