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Review

# The multiple faces of CXCL12 (SDF-1$\alpha$) in the regulation of immunity during health and disease

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## ABSTRACT

**Chemokines are a group of small, structurally related molecules that regulate the trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane G-protein-coupled receptors. As key chemoattractants of inflammatory leukocytes, chemokines have been marked as potential targets for neutralization in autoimmune diseases. Cancer cells also express chemokines, where they function as survival/growth factors and/or angiogenic factors that promote tumor development and angiogenesis. Accordingly, these functions make them attractive targets for therapy of these diseases. Recently, we reported that one of these chemokines CXCL12 (SDF-1$\alpha$) functions as an anti-inflammatory chemokine during autoimmune inflammatory responses and explored the mechanistic basis of this function. As a pleiotropic chemokine, CXCL12 participates in the regulation of tissue homeostasis, immune surveillance, autoimmunity, and cancer. This chemokine is constitutively expressed in the BM and various tissues, which enables it to regulate the trafficking and localization of immature and maturing leukocytes, including BM stem cells, neutrophils, T cells, and monocytic cells. We have shown recently that CXCL12 increases immunological tolerance in autoimmune diseases by polarizing Tregs and by doing so, restrains the progression of these diseases. This finding suggests a possible use of stabilized rCXCL12 as a potential drug for therapy of these diseases and targeted neutralization of CXCL12 for therapy of cancer diseases. The current review explores the different biological properties of CXCL12 and discusses the implications of CXCL12-based therapies for autoimmunity and cancer diseases. *J. Leukoc. Biol.* 88: 463–473; 2010.**

## Introduction

Chemokines are a group of small (8–14 kDa), structurally related molecules that regulate the trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane G-protein-coupled receptors [1–3]. Chemokines play fundamental roles in the development, homeostasis, and function of the immune system [1–3]. Although chemokines are produced primarily by immune cells, they are also produced by nonimmune cells and affect their biological function(s). For example, vascular endothelial cells produce chemokines that are involved in angiogenesis [1–3].

Based on the arrangement of the conserved cysteine residues, the chemokines are divided into four subgroups: C, CC, CXC, and CX3C [1–3]. The two major subgroups are the CXC and CC subgroups, in which the two cysteines are separated by a single amino acid (CXC) or are adjacent to each other (CC). The CXC chemokines are subdivided further into ELR and non-ELR types, according to the presence or absence of a ELR tripeptide sequence adjacent to the CXC motif [1–3].

Much attention has been paid to the key role of chemokines in inflammatory processes [4–10] and especially, inflammatory autoimmune diseases, such as MS and its experimental models [6, 8, 11–22], T1DM [23, 24] and RA [25–27], amongst others. As key chemoattractants of inflammatory leukocytes, chemokines have been marked as potential targets for neutralization in autoimmune diseases [6, 8, 11–22, 24–27]. Some of the results of these studies have been applied to human patients, thus far with limited success.

EAE is a T cell-mediated autoimmune disease of the CNS, and animals in which EAE is induced are used as an experimental model for MS. One of the chemokines that is likely to participate in the regulation of EAE is CXCL12 (SDF-1$\alpha$). This is a pleiotropic chemokine that participates in the regulation of tissue homeostasis, immune surveillance, inflammatory responses, and cancer development. Under noninflammatory conditions, CXCL12 attracts leukocytes to the CNS as a part of

Abbreviations: BM=bone marrow, EAE=experimental autoimmune encephalomyelitis, ELR=Glu-Leu-Arg, Foxp3=forkhead box p3, HEV=high endothelial venule, MS=multiple sclerosis, RA=rheumatoid arthritis, SDF-1$\alpha$=stromal cell-derived factor 1$\alpha$, T1DM=type I diabetes mellitus, Thnp=nonpolarized CD4$^+$ T cell(s), Tr-1=regulatory T cell I, Treg=regulatory T cell

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the immune surveillance of tissues, thereby functioning as a "proinflammatory" chemokine. In a recent study, we showed that when leukocytes rapidly enter a site of inflammation in the CNS during ongoing EAE, this chemokine shifts its potential proinflammatory function to become an anti-inflammatory chemokine [28]. This change skews the polarization of $CD4^+$ antigen-specific effector T cells (also known as Th1 and Th17 cells), which normally promote inflammation, so that they become antigen-specific Tregs that produce a key anti-inflammatory cytokine, IL-10 [28], and restrain the development and progression of the disease. In light of these findings, the present article reviews the multiple faces of CXCL12 (SDF-1$\alpha$) in the regulation of immunity during health and disease and their therapeutic implications for cancer and autoimmunity.

## THE ALTERNATIVE ROLE OF CHEMOKINES IN TISSUE HOMEOSTASIS, IMMUNE SURVEILLANCE, AUTOIMMUNITY, AND CANCER

Chemokines direct cellular movement and relocalization, both of which are essential for many fundamental physiologic processes, which include embryonic development, neovascularization and angiogenesis, immunologic responses, wound healing, and organ repair. In embryonic life, together with other chemoattractive growth factors, CXCL12 directs the proliferation and differentiation of immature progenitor cells [29] by activation of the adhesion machinery, cytoskeleton rearrangement, the control of cell cycle, and the secretion of proteolytic enzymes [29]. In adults, chemokines have three major functions: They control tissue homeostasis; they direct the development of inflammatory responses, including inflammatory autoimmune diseases; and they function as growth/survival factors and as chemoattractants for cancer cells to support metastasis. For tissue homeostasis, chemokines are involved closely in the regulation of cell migration, particularly leukocytes, from the BM to blood and tissues, to ensure a dynamic balance of cell number and type in these organs under diverse conditions. For example, the interaction between the CC chemokine receptor, CCR2, which is expressed predominantly on BM-derived monocytes, and its ligands, particularly CCL2, is essential for directing the rapid recruitment of $CCR2^+$ cells from the BM to a site of inflammation, where they are required to promote inflammatory processes [30]. Thus, CCR2-deficient mice are highly resistant to the induction of inflammatory autoimmunity [20]. Another example for the role of CXCL12 in tissue homeostasis is its constitutive expression in the BM and other tissues, which include the skin and heart, epithelial cells in human bile ducts, and brain endothelium [29]. This constitutive expression is responsible for regulating the trafficking and localization of immature and maturing leukocytes to these tissues. During steady-state conditions, these cells egress continuously from the BM reservoir to the circulation to replenish the blood with new cells throughout the lifespan of the individual [31]. In addition to its role in regulating BM stem cell homeostasis, CXCL12 is involved directly in neutrophil homeostasis under normal and stress conditions. The number of circulating neutrophils is tightly regulated to ensure adequate protection of the host against microbial pathogens and concomitantly minimizing damage to noninflamed tissues. Neutrophil homeostasis in the blood is achieved by balancing neutrophil production, neutrophil release from the BM, and neutrophil clearance from the circulation. Accumulating evidence suggests that signaling by CXCL12, through its major receptor CXCR4, plays an important role in maintaining this homeostasis [32]. Very recently, Eash et al. [33] generated mice with a myeloid lineage-restricted deletion of CXCR4 to define the underlying mechanisms of CXCR4 signaling in neutrophil homeostasis. Similar to its effects on other leukocytes, particularly monocytes and T cells, CXCL12 directs the migration of neutrophils to organs, particularly immune-privileged organs, as part of the immune surveillance of the body. Based on observations that have been made in experimental models of autoimmune disease, whose results have been extended to humans, it is believed that the interaction between antigen-specific effector T cells and their target autoimmune antigen in T cell-mediated inflammatory autoimmune disease following tolerance breakdown begins with the activation of HEVs. Their activation then facilitates the extravasation of large numbers of lymphocytes and monocytes from the blood to the tissues to initiate the autoimmune process [34]. Inflammatory chemokines possess three key complementary features in this process: They attract various types of leukocytes, particularly lymphocytes, monocytes, and neutrophils to the inflammatory site; they activate adhesion molecules on HEV and leukocytes to allow rapid influx of cells to the autoimmune site [35–40]; and they polarize $CD4^+$ T cells directly to become effector proinflammatory cells [26, 41]. Thus, not only inflammatory chemokines but also the key adhesion receptors that they activate, namely the $\alpha4\beta1$-integrin, VLA-4, have become a major target in the therapy of MS and other autoimmune diseases [42–44].

The role of chemokines in tumor biology is complex. It appears that many tumor cells produce a large variety of chemokines and their receptors [45–47]. The actions of chemokines in tumor biology can be divided into direct autocrine effects, in which tumor cells produce a given chemokine and its target receptor, and indirect effects, such as recruitment of tumor-associated macrophages, to support tumor development and angiogenesis [45, 48–51]. On their autocrine effects, chemokines function as growth/survival factors for cancer cells, as proangiogenic factors, and as chemoattractants for cancer cells to support metastasis. At least three major chemokines, CXCL8, CXCL12, and CCL2, all of which are produced by various solid tumors, such as malignant melanoma, liver and pancreatic tumors, colon carcinoma, breast cancer, and prostate cancer, function as autocrine growth factors [45–47]. It has been proposed that CXC chemokines with the ELR tripeptide sequence immediately adjacent to the amino-terminal of the CXC motif ($ELR^+$ CXC chemokines), such as CXCL8, are angiogenic and that $ELR^-$ CXC chemokines, such as CXCL10, are angiostatic [1–3]. Other data have shown that the $ELR^-$ CXC chemokine, CXCL12, can act as a direct chemoattractant for endothelial cells in vitro and as an angiogenic factor in vivo [52–54]. CXCL12 is also thought to play a key role in at-

tracting tumor cells to their target organs to facilitate the formation of metastasis, such as the attraction of prostate cancer cells to the bones [55, 56]. On the indirect effects of chemokines, chemokines, such as CCL2, are thought to be highly important for attracting $CCR2^+$ monocytic cells that assist tumor development, rather than destroying tumors, by producing angiogenic factors at the tumor site and producing cytokines, particularly IL-10, that suppresses anti-tumor immunity [45, 48, 49, 51, 57–62].

## THE MAJOR PARADOX OF CHEMOKINES AS TARGETS FOR THERAPY OF INFLAMMATORY AUTOIMMUNE DISEASES

The underlying idea of antichemokine therapies for inflammatory autoimmune disease is that targeted neutralization of chemokines would restrain the autoimmune response by inhibiting the influx of inflammatory leukocytes to a site of autoimmune-induced inflammation. Reviewing the results of the many studies that have used this strategy reveals a major paradox. Although most of the 50 known chemokines can direct the migration of the same leukocytes, targeted neutralization of only one chemokine, such as CCL2, CCL3, CCL5, or CXCL10, is sufficient to suppress the entire inflammatory process [8, 20, 21, 41, 63–67]. Therefore, the question that begs an answer is why other chemokines that also attract the same type of leukocyte to the autoimmune site do not compensate for the absence of this single chemokine. In addition, it is not clear why neutralization of as few as eight to 10 of the 50 different chemokines can effectively suppress the attacks in autoimmune inflammatory diseases [8, 20, 21, 41, 63–67]. Hence, what are the attributes of this limited number of chemokines that make them so important in the regulation of inflammatory processes?

A partial explanation for this paradox could be that these chemokines have other biological actions that are associated with these autoimmune inflammatory diseases, as well as being chemoattractants. For example, it has been shown recently that CCR5 ligands are essential for the induction of costimulatory signals via stiumulation of the CCR5 receptor on target effector $CD4^+$ T cells and that these signals are essential to promote IL-2-dependent activation of these cells [68–70]. This may explain why targeted neutralization of CCR5 ligands is so effective in suppressing different autoimmune diseases, such as EAE [65, 71, 72], RA [25, 73], and T1DM, in different strains of mice [24].

## EFFECTOR $CD4^+$ T CELL SUBSETS

In 1986, Robert Coffman and Timothy Mosmann were the first to describe the division of $CD4^+$ T cells into functional subsets based on cytokine production, which they termed Th1 and Th2 cells [74, 75]. Subsequently, other types of effector T cell and Treg subsets were identified. It has also been shown that during their activation, Thnp undergo epigenetic changes when differentiating into different subtypes. These epigenetic changes are dependent on the cytokine milieu that is present within the microenvironment, in which they are undergoing clonal expansion [76, 77]. For example, in the presence of high levels of IL-12 and IL-18 and low levels of IL-4, Thnp will be polarized preferentially into IFN-$\gamma^{high}$IL-$4^{low}$-producing Th1 cells that also produce substantial levels of IL-2, TNF-$\alpha$, and other proinflammatory cytokines [78–81]. On the other hand, a milieu that is enriched with IL-4 and has low levels of IL-12 and IL-18 will drive the polarization of Thnp to become IL-4-producing Th2 cells [82, 83]. Although Th1 cells promote the inflammatory process, Th2 cells are associated with helping B cells switch their isotype and change their biological function [82, 83]. Thus, from the practical perspective, these observations suggest that anti-IL-12 and/or anti-IL-18 therapies may be beneficial in inflammatory autoimmunity. Indeed, the administration of neutralizing antibodies to IL-12 or IL-18 could suppress several experimentally induced autoimmune diseases effectively by shifting the Th1/Th2 cell balance in favor of Th2 cells [81, 84–87]. The drawback of this therapeutic strategy is the danger of substituting a Th1-driven inflammatory process with a Th2-directed allergic response [88].

A third subtype subtype of $CD4^+$ effector cell is the recently discovered IL-17-producing Th17 cell [89–93]. These cells are polarized initially by IL-6 and TGF-$\beta$ and thereafter, by IL-21 in an autocrinic way via the STAT3 pathway. This initial stepwise polarization increases their susceptibility to IL-23, which augments their rapid polarization [89–93]. IL-23-deficient mice are EAE-resistant, and therapies that neutralize this cytokine suppress EAE and other T cell-mediated autoimmune diseases [93–100]. It is also believed that Th1 and Th17 cells contribute independently to $CD4^+$-directed inflammatory responses that include T cell-mediated autoimmunity by different mechanisms [99, 100].

Are there other subtypes of effector $CD4^+$ T cells yet to be identified? The basic concept, which was proposed by Mosmann and colleagues, is that the Thnp could be divided into two exclusive types of effector cells, namely the IFN-$\gamma^{high}$IL-$4^{low}$ Th1 cells and the IL-$4^{hig}$IFN-$\gamma^{low}$ Th2 cells, and that the lineage characteristics of each subtype are stable for as long as 20 years. Hence, the discovery of other types of effector T cells, such as Th17 cells [89–93], and the recent observations that challenge the dogma that these subsets are stable [101–103], which is discussed later in this review, have unwittingly opened a Pandora's box of complexity and controversy. As for other effector T cell subtypes, two new subtypes have been discovered recently: one subtype that produces IL-22 (Th22 cell) predominantly and a second subtype that produces IL-9 (Th9 cells) [104, 105]. Future investigations may well lead to the discovery of other new effector T cell subtypes.

## TREG SUBSETS

Despite major functional differences among Th1, Th17, and Th2 cells, all are considered to be effector T cells that can promote inflammatory responses (Th1 and Th17 cells) or B cell-directed immunity (Th2 cells). What regulates their activities? As mentioned already, the $CD4^+$ T cell subsets that control the function of effector T cells are known as Tregs, and

these subsets can be divided into two major categories: those that express Foxp3 (Foxp3$^+$) and are known as natural Tregs [106, 107] and the antigen-specific Tregs, which are Foxp3$^-$ and suppress the function of effector T cells by producing suppressor cytokines, such as TGF-$\beta$ [108, 109], IL-10 [110], and possibly IL-35 [111]. Foxp3$^+$ Tregs were discovered by Shimon Sakaguchi [106], who showed that their depletion leads to the spontaneous development of various autoimmune diseases, which could be reversed by adoptive transfer of natural Tregs from naïve donors. The relevance of these findings has been extended to humans. Similar to the T cell subsets in rodents, the human T cell subsets also include a significant portion of CD4$^+$CD25$^+$Foxp3$^+$ T cells that functions as suppressor Tregs. Interestingly, human subjects who carry a functional mutation in the *FOXp3* gene spontaneously develop a severe autoimmune disorder that is called the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome [112] and resembles the syndrome that develops when mice are depleted of CD4$^+$CD25$^+$Foxp3$^+$ T cells [113]. For Foxp3$^-$ Tregs, two major subtypes have been identified thus far. Almost 20 years ago, Howard Weiner and his group [108, 109] identified a novel type of antigen-specific Tregs that produces large amounts of the suppressor cytokine/growth factor TGF-$\beta$ and small amounts of other cytokines and subsequently, named them Th3 cells. Weiner and his group [108, 109] showed that these Th3 cells seem to direct immunological tolerance within the gut, and their function could be induced by oral tolerance. In 1977, Groux et al. [110] identified another type of antigen-specific Tregs that produces mostly IL-10 and designated them as Tr-1 cells. It is believed that these Tr-1 cells are essential in the maintenance of active tolerance and in particular, for restraining the harmful activity of autoimmune T cells [114].

## FLEXIBILITY IN THE POLARIZATION OF CD4$^+$ T CELL SUBSETS

The traditional, consensual opinion about the mechanism of T cell polarization into subsets assumed that epigenetic changes lead to a well-defined characteristic of each CD4$^+$ T cell subset with relative inflexibility [115]. For example, during their antigen-specific T cell activation, Thnp are polarized into Th1 cells in the presence of high levels of IL-12 and low levels of IL-4 [78]. Under these conditions, IL-12 activates a specific signal transduction cascade that leads to the synthesis of a T-box transcription factor, which controls the expression of the hallmark Th1 cytokine, IFN-$\gamma$ [116]. According to the traditional dogma, these polarized CD4$^+$ T cells would continue producing their typical cytokine profile as long as they continue to proliferate in response to their target antigen. For example, proliferating effector Th1 cells would continue producing high levels of IFN-$\gamma$ and TNF-$\alpha$ and low levels of IL-4 as long as they are activated. Thereafter, these cells undergo apoptosis or become long-term memory cells [117]. Once becoming long-term memory cells, they arrest their typical cytokine production and enter a resting phase. Upon reactivation, it is believed that upon reactivation, they "remember" their basic cytokine profile that they obtained during their initial polarization and continue producing this cytokine profile. Other examples are: Th2 cell polarization, which is directed initially by IL-4-induced signaling via the STAT6 and STAT5 transcription factors [118] and then by the GATA-3 transcription factor [119], and Th17 cells, which are polarized initially by IL-6 and TGF-$\beta$ and thereafter, in an autocrinic way by IL-21 via the STAT3 pathway and the major nuclear orphan receptor ROR$\gamma$t [120], and the resultant polarization increases their susceptibility to further polarization by IL-23 [89–93].

For Foxp3$^+$ Tregs, the key transcription factor that characterizes the CD4$^+$CD25$^+$ Tregs is Foxp3, which is essential for their normal development and function [106, 121–123]. The dominant cytokine that drives the polarization of Foxp3$^-$ to Foxp3$^+$ CD4$^+$ T cells is TGF-$\beta$ [124, 125]. Less is known about the signal transduction events by which Foxp3$^-$ IL-10-producing Tr-1 cells are polarized in the presence of IL-10 and IL-2 [110]. A recent study showed that this polarization is directed in human Tregs through a STAT5-responsive intronic enhancer in the IL-10 locus [126].

The results of several recent studies have now provided evidence that challenges the traditional dogma, which suggests that CD4$^+$ T cells undergo epigenetic changes during T cell polarization and display limited flexibility. In 2007, Anderson et al. [101] showed that IFN-$\gamma$-producing Th1 cells could be repolarized into IL-10-producing Tr-1 cells in chronic, cutaneous leishmaniasis (see also a recent review by O'Garra and Vieira [103]). A more recent example is the role of another cytokine, IL-27, in the polarization of CD4$^+$ T cells. Together with IL-12, IL-27 can polarize Th1 cells [127–129], and then together with IL-6, it can transform polarized Th1 cells, Th2 cells, and even Th17 cells into IL-10-producing Tr-1 cells [130]. These effects are dependent on the transcription factors STAT1 and STAT3. The polarization of CD4$^+$ T cells into Th1 cells by IL-12 and IL-27 is dependent on STAT1 and STAT3, whereas the transformation of polarized Th1 cells by IL-6 and IL-27 is dependent on STAT3 [130–132]. Interestingly, no cytokines that are capable of reversing polarized Tr-1 cells into effector Th1 or Th17 cells have been found so far. We have shown recently that flexibility in skewing Th1 to Tr-1 could also be induced by CXCL12 via stimulation of the CXCR4 receptor on Th1 cells [28].

## THE ROLE OF CHEMOKINES IN DIRECTING CD4$^+$ T CELL POLARIZATION AND FUNCTION

The role of chemokines in directing the biological function of CD4$^+$ T cells can be viewed from two perspectives: the differential effect of chemokines on the biological functions of polarized T cells and the ability of chemokines to polarize CD4$^+$ T cells directly into selective subsets. Of the two roles, more is known about the role of chemokines on directing the biological properties of polarized T cells. In 1997, Sallusto and coworkers [133] showed that Th2 cells preferentially express the chemokine receptor, CCR3. In the following year, the same group extended this study to show that Th1 cells also express CXCR3, and Th2 cells preferentially express the chemokine receptors, CCR3 and CCR4 [134]. Th1 and Th2 cells express

CCR5 [133–135]. Subsequently, Hirahara et al. [136] reported that Foxp3$^+$ Tregs also preferentially express CCR4. The direct consequence of these findings refers to the selective homing of various cell types as a result of their chemokine receptor expression. For example, Th17 cells predominantly express the chemokine receptor, CCR6, and its exclusive ligand CCL20 is constitutively expressed in the epithelial cells of choroid plexus in mice and humans to direct the homing of Th17 cells [137]. The results of a recent study have shown that an attenuated form of disease develops as a result of aberrant trafficking of Th17 cells when EAE is induced in CCR6-deficient mice [137]. The consequences of the CCL20-CCR6 interaction on the biological properties of Th17 cells are not known yet and still need to be studied. Another example is the CXCR3 ligand, CXCL10, which is preferentially expressed in the CNS during the accelerating phase of EAE and is associated with enhanced recruitment of CXCR3$^+$ Th1 cells to the CNS [21].

In addition to directing the migratory properties of CD4$^+$ T cells, the selective interaction of chemokines and their receptors affects their biological properties. For example, the chemokine receptor, CCR8, is preferentially expressed on CD45$^+$ Th2 cells and Foxp3$^+$ Tregs [138], and Foxp3$^+$ Tregs suppress effector T cell functions by various mechanisms [107], and Th2 cells produce IL-4, which also restrains Th1 activities [80]. It has been suggested that that Th2 and Foxp3$^+$ T cells are not only attracted to the site of inflammation via the CCR8 receptor but also, that the interaction of CCL1 with its exclusive CCR8 receptor potentiates their anti-inflammatory activities [138], although this possibility has yet to be explored. Another example is the interaction between the CCR5 receptor that is expressed on Th1 cells and Th2 cells and its three different ligands, CCL3 (MIP-$\alpha$), CCL4 (MIP-$\beta$), and CCL5 (RANTES) [1]. Recently, Molon et al. [68] reported that signals that are mediated by CCL5 by stimulation of the CCR5 receptor are required for effective activation of the CCR5 receptor, thus serving as costimulatory signals in T cell activation. Subsequently, Camargo et al. [70] showed that the interaction between the CCR5 receptor and its ligands induces signals via translocation of the transcription factor, NFAT, which results in IL-2 production by CD4$^+$ T cells.

Less is known about the role of chemokines in the polarization of CD4$^+$ T cells. CXCR3 is a chemokine receptor that is expressed mainly on CD4$^+$ cells and in particular, Th1 cells [134], and three ligands bind to this receptor: CXCL9 (monokine induced by IFN-$\gamma$), CXCL10 (IFN-inducible protein 10), and CXCL11 (IFN-inducible T cell-$\alpha$ chemoattractant). In 1998, Gangur et al. [139] reported that the addition of CXCL10 to proliferating human T cells polarizes them into IFN-$\gamma^{high}$IL-4$^{low}$ Th1 cells. Based on this information, we have shown that indeed, CXCL10 not only attracts CXCR3$^+$ T cells to the site of inflammation but also polarizes them into effector Th1 cells in two different experimental models of inflammatory autoimmune disease, namely EAE and adjuvant-induced arthritis [26, 41]. Thus, targeted neutralization of CXCL10 reduces the severity of ongoing EAE and adjuvant-induced arthritis by shifting the Th1/Th2 cell balance in favor of the Th2 cells [26, 41]. More recently, Flanagan et al. [140] reported that the lymphoid chemokine, CCL21, polarizes naïve T cells into Th1 cells. In our recent publication, we reported that chemokines may polarize some CD4$^+$ subsets, and this finding supports the notion that competence is not limited only to effector cell polarization but also for skewing the polarization of effector T cells into Tregs [28].

## THE MULTIPLE FACES OF CXCL12 DURING ONGOING INFLAMMATORY AUTOIMMUNITY

The CXC chemokine, CXCL12, which is also known as SDF-1 [141], was identified originally as a growth factor for murine pre-B cells [142]. It exists in two alternative splice variants: SDF-1$\alpha$ and SDF-1$\beta$. It is expressed constitutively by various cells and exhibits chemoattractive activity for monocytes, BM neutrophils, and early-stage B cell precursors, as well as being a highly efficient and potent chemoattractant for T cells and a costimulator of their activation [143]. Furthermore, CXCL12 induces adhesion of T cells to ICAM-1 (CD54) [144] by up-regulating the binding activity of LFA-1 (CD11a/CD18) and modulates the adhesion of $\alpha$4-$\beta$7 integrin-mediated lymphocytes to mucosal addressin cell adhesion molecule-1 and fibronectin [145]. In light of these findings, it is thought that CXCL12 plays an important role in the attraction of T cells to specific sites. Furthermore, it was also suggested that CXCL12 could play a proinflammatory role in various autoimmune diseases, particularly RA and nephritis, in murine lupus erythematosus and therefore, could be a valid target for neutralization in these diseases [35, 146]. Our findings showing that CXCL12 functions as an anti-inflammatory chemokine during the inflammatory process challenge this concept [28].

In the healthy CNS, CXCL12 serves as a survival and migratory factor for neural and oligodendrocyte precursors that express the CXCR4 receptor [147]. The expression of CXCL12 within the CNS is up-regulated in the astrocytes of the MS brain; hence, its role in the regulation of this disease is not fully understood [148–150].

Our working hypothesis has been that targeted neutralization of CXCL12 during EAE would suppress the disease. In initial experiments, we tried to neutralize CXCL12 at different time-points after active EAE was induced and found contradictory effects. When a CXCL12-Ig fusion protein was administered to mice after the induction but before the onset of symptoms of EAE, we showed that the onset of disease was delayed for 2–3 days (Moran Meiron, Yaniv Zohar, and N. Karin, unpublished data). In contrast, there was rapid remission of the disease when the fusion protein was administered after the onset of disease [28].

The delay of the onset of disease following early administration of the CXCL12-Ig fusion protein could be explained by CXCL12 acting as a chemoattractant of leukocytes that are essential for the regular "policing" of the CNS. It is likely that endogenous CXCL12, which is produced within the CNS by astrocytes and other cells, is involved in modulating the migration of those leukocytes that are essential for the regular policing of the CNS. The development of progressive, multifocal leukoencephalopathy following anti-$\alpha$4-integrin (VLA-4) ther-

apy [151] may serve as an example for the importance of policing the CNS by T cells and macrophages.

However, according our observations, when the inflammatory process within the CNS later enters into an accelerating phase, CXCL12 functions as an anti-inflammatory chemokine that directs the polarization of $CD4^+$ T cells and macrophages to become IL-$10^{high}$-producing Tregs [28]. The implication of these results for humans is that CXCL12 could be used as a potential drug only during advanced stages of inflammatory autoimmunity because of its pleiotropic characteristics.

The results of several studies have shown that C-C chemokines may affect the polarization of antigen-specific T cells by altering the Th1/Th2 cell balance [6, 152, 153]. However, the results of our recent study have shown that a chemokine can direct the polarization of IL-10-producing Tregs [28], thereby introducing a new concept that proposes chemokines as potential anti-inflammatory proteins. Are there other chemokines that polarize Tr-1 cells? We have observed recently that of the three CXCR3 ligands, CXCL9, CXCL10, and CXCL11, only CXCL11, which competes effectively with CXCL10 in binding their common CXCR3 receptor, antagonizes the function of the other CXCR3 ligands by skewing $CD4^+$ T cell polarization into IL-10-producing Tr-1 cells (Yaniv Zohar, Gizi Wildbaum, Nir Netzer, and N. Karin, manuscript in preparation). CXCR3-deficient mice can develop an extremely severe form of EAE and T1DM [154, 155]. We do not exclude the possibility that the exacerbation of these diseases in experimental models is, in part, a result of the lack of protective, anti-inflammatory signals, which are mediated by the CXCL11-CXCR3 interactions.

## CXCL12 POLARIZES ANTIGEN-SPECIFIC TR-1 CELLS BY TWO COMPLEMENTARY MECHANISMS

Antigen-specific Tr-1 cells were first identified by Groux et al. [110], who showed that the addition of IL-10 and IL-2 to cultured, primary T cells caused them to proliferate in response to their target antigen and polarize them into Tr-1 cells. Under these working conditions (in vitro) and at an inflammatory site (in vivo), IL-10 is produced by two major types of cells: the APCs that include dendritic cells (also known as D2 cells) and monocytes (also known as M2 cells) [61, 156] and the IL-10-producing Tr-1 cells [110]. We have shown that CXCL12 via stimulation of the CXCR4 receptor induces IL-10 production in macrophages and also acts directly on $CD4^+$ T cells that are being activated. We, therefore, suggest that the outcome of CXCL12-induced skewing of $CD4^+$ T cells to become Tr-1 cells is a result of two complementary pathways: a direct effect on the $CD4^+$ T cells and an indirect effect that is mediated by antigen-presenting T cells to produce IL-1, which together with IL-2, polarizes Tr-1 cells (**Fig. 1**).

## IMPLICATIONS FOR THERAPY OF AUTOIMMUNITY, GRAFT REJECTION, AND CANCER

Various tumors and in particular, androgen-dependent tumors, such as prostate cancer, breast cancer, and ovarian cancer cells,

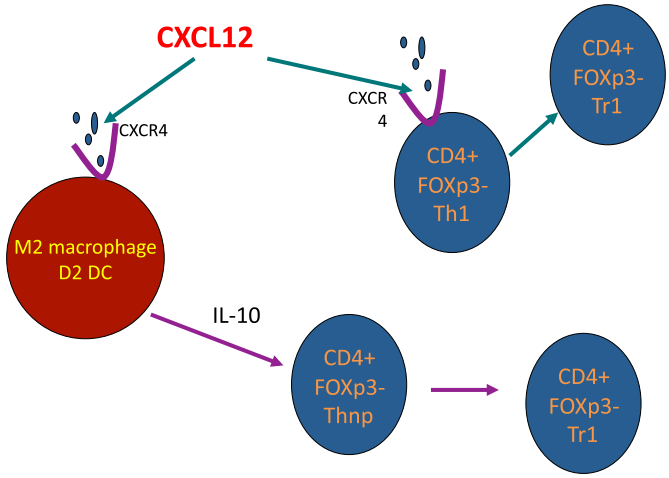


**Figure 1. Direct and indirect polarization of Tr-1 by CXCL12.** CXCL12 polarizes Tr-1 cells by two complementary independent mechanisms. A direct effect on $CXCR4^+$ $CD4^+$ T cells, including effector Th1 cells, and an indirect effect by eliciting IL-10 production in M2 macrophages and D2 dendritic cells (DC) that then polarize antigen-specific Tr-1.

produce CXCL12 predominantly and express its two receptors, CXCR4 and CXCR7 [157–161]. In these types of tumors, CXCL12 functions, in an autocrinic manner, as a survival/growth factor, as well as a chemoattractant of tumor cells, and in particular, during tumor spread to the bones [53, 158, 159, 162–166]. Aside from its role as a survival/growth factor and a chemoattractant of tumor cells, CXCL12 suppresses anti-tumor immunity by promoting IL-10 production by $CXCR4^+$ tumor-associated macrophages that are recruited at the tumor site. The relevance of this hypothesis has been explored in humans by Zou et al. [157], who showed that CXCL12 recruits and directs the function of plasmacytoid precursor dendritic cells so that they become IL-10-producing cells that are capable of suppressing immunity in human ovarian neoplasms. These results reinforce the idea that anti-CXCL12-based therapies could be beneficial for some types of cancers.

As mentioned previously, CXCL12 is essential for the homing of BM stem cells to the BM, and this action has potential implications for the treatment of some types of cancer. The ability of CXCL12 to attract BM-derived cells to the BM is mediated by the CXCR4 receptor [31]. Thus, a molecule that selectively blocks the CXCR4 receptor (AMD3100, Pleixafor) has been approved recently as a drug (in combination with GM-CSF) for hematopoietic stem cell mobilization from the BM to the blood, where they could be collected for autologous human stem cell transplantation [167]. This approach has been approved for intervention in two relevant cancer diseases: multiple myeloma and non-Hodgkin's lymphoma [167].

As discussed above, CXCL12 has pleiotropic actions in inflammatory autoimmune disease. It directs monocyte and T cell recruitment to restricting sites for immune surveillance, and by doing so, it stimulates effector T cells to combat potential infections at these immune-restricted sites. Nevertheless, it acts in an opposing manner during severe inflammation, where it polarizes IL-10-producing Tregs and IL-10-producing macrophages to suppress

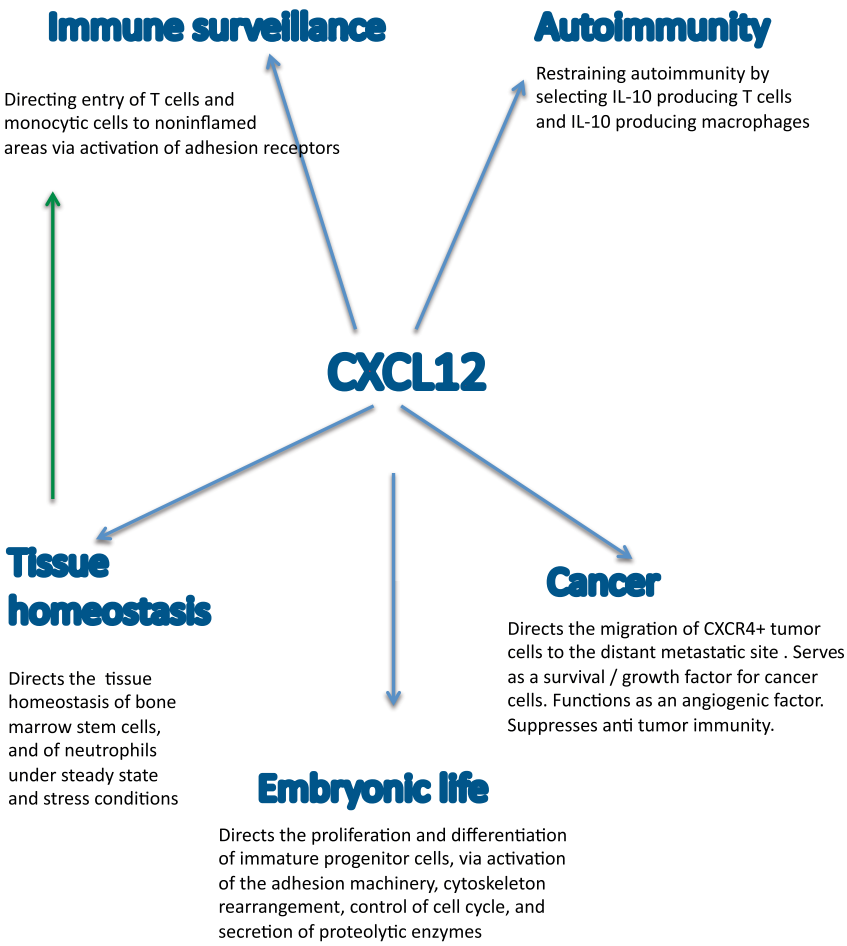


**Figure 2 . Multiple faces of CXCL12.**

the inflammatory process [28]. This finding suggests that CXCL12-based therapies for autoimmune diseases may be considered in the treatment of ongoing diseases but not for prophylactic use.

## THE MULTIPLE FACES OF CXCL12 IN TISSUE HOMEOSTASIS, IMMUNE SURVEILLANCE, AND CANCER: WHAT SHOULD BE TAKEN IN CONSIDERATION WHEN PLANNING LONG-TERM THERAPEUTIC INTERVENTIONS?

The aims of administering a CXCL12-Ig fusion protein for treating inflammatory autoimmunity are twofold: to direct the polarization of antigen-specific T cells so that they become IL-10-producing Tr-1 cells and to polarize macrophages into IL-10-producing macrophages. The short half-life of chemokines suggests that a stabilized form of CXCL12, such as CXCL12-Ig- fusion protein, could be used in the therapy of inflammatory autoimmunity [28]. Which of the inflammatory autoimmune diseases could be a suitable candidate for CXCL12-Ig-based fusion protein therapy? Based on the underlying mechanism of disease, autoimmune diseases can be separated into those in which the direct attack of self-components is mediated by effector T cells, such as MS; inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, T1DM, amongst others; and antibody-mediated autoimmunity, such as systemic lupus erythematosus. Tentatively, CXCL12-based therapy should be preferentially directed against effector T cell-mediated autoimmunity. Hence, it should be noted that effector T cells also direct and assist B cells to produce autoantibodies in antibody-directed autoimmunity. Thus, CXCL12-Ig-fusion protein therapy could also be beneficial for these diseases.

Other points that should not be overlooked are the other activities of this pleiotropic chemokine, which includes its role in tissue homeostasis, immune regulation of cancer, and immune surveillance. As mentioned previously, the major functions of CXCL12 in the maintenance of tissue homeostasis are directing the homing of BM stem cells and neutrophil homeostasis to ensure adequate protection against microbial pathogens [32]. Whether repeated administration of a CXCL12-Ig fusion protein would affect these functions has not yet been established. Another point of concern is whether this type of therapy would affect the Yin-Yang regulation of autoimmunity and cancer, in which the immune system protects the host from pathogens and emerging cancer cells and at the same time, minimizes local damage to tissue. Thus, shifting the balance between effector response and tolerance to favor increased tolerance to self-antigens may promote unwanted tolerance to tumor growth [168, 169]. Soluble peptide therapies [170–172] can be used to overcome this obstacle, as they can induce antigen-

specific tolerance, which could then be amplified by CXCL12-Ig fusion protein therapy. It should be noted, however, that such therapy might also result in undesirable side-effects, such as causing the production of antigen-specific IgE, which in turn, can lead to an allergic response [88].

## CONCLUSIONS

This review focuses on exploring the various biological properties of CXCL12 as summarized in **Fig. 2**. In particular, its role in directing and regulating immunity. In this review, we describe the distinct functions of this chemokine in noninflammatory/low-inflammatory conditions and severe inflammation. In noninflammatory or low-inflammatory conditions, CXCL12 attracts monocytic cells and T cells to various tissues as a part of immune policing. In severe inflammation and at tumor sites, CXCL12 functions as an anti-inflammatory chemokine that skews the polarization of antigen-specific Tregs and IL-10-producing dendritic cells/monocytic cells to restrain the inflammatory process in inflammatory diseases [28] and suppress anti-tumor immunity in cancerous diseases [157]. Although these functions are beneficial for combating autoimmunity or restraining an aggressive, infectious, inflammatory process, they are undesirable in cancerous diseases, as they suppress immunity against cancer cells. It is therefore likely that therapies aiming at neutralizing the biological function of CXCL12 could be used in the therapy of various cancer diseases, whereas the administration of stabilized CXCL12 could be considered for therapy of ongoing inflammatory autoimmune diseases.

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