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Review

The dual roles of inflammatory cytokines and chemokines in the regulation of autoimmune diseases and their clinical implications

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ABSTRACT

Cytokines and chemokines are secreted, small cell-signaling protein molecules, whose receptors are expressed on immune cells. These factors play a critical role in immune cell differentiation, migration, and polarization into functional subtypes and in directing their biological functions. Much attention has been devoted to exploring the role of key inflammatory cytokines and promigratory chemokines in autoimmune, autoinflammatory, and allergic diseases, leading to development of therapeutic strategies that are based on their targeted neutralization. Recent studies, including those coming from our groups, show that several major proinflammatory cytokines and chemokines, including IFN-γ, IL-2, CCL2, and CXCL12, may also function as anti-inflammatory mediators and therefore, may have potential as anti-inflammatory drugs. Likewise, major anti-inflammatory mediators, such as TGF-β, may under certain conditions, in combination with other cytokines, exhibit proinflammatory function and direct the polarization of the highly inflammatory CD4$^+$ Th17 cells. We show here that the biological function of pro- and anti-inflammatory cytokines is dependent on three key parameters: the local concentration of a given cytokine, the stage of disease in which it is administered, and its combination with other cytokines. The therapeutic implications of these findings are discussed, including two very recent studies summarizing clinical trials, in which low-dose administration of IL-2 was used to successfully suppress HCV and GVHD. *J. Leukoc. Biol.* 93: 51–61; 2013.

Introduction

Cytokines are secreted, small molecules that modulate the immune response and have an essential role during T cell differentiation. It was believed previously that each cytokine exerts immune stimulatory (inflammatory) or inhibitory (anti-inflammatory or regulatory) activities. For historical reasons, as regulatory "suppressor" cells were not universally acknowledged for many years, attention has been mainly devoted to the proinflammatory activities of cytokines. The extensive characterization of Tregs, which suppress Teff functions by several mechanisms, among them, suppressor cytokine production [1, 2], awakened interest in the role of cytokines as regulatory mediators. Recent studies challenge the traditional concept of dividing CD4$^+$ T cells into reciprocal Teff and Treg populations and demonstrate plasticity in switching from one type to the other [3, 4]; in addition, these studies demonstrate that multiple cytokines can mediate inhibitory and stimulatory effects on the immune system.

Chemokines are small (8–14 kDa), structurally related chemotactic cytokines that regulate cell trafficking through interactions with specific seven-transmembrane GPCRs. They are thought to be critical players in the development of inflammatory diseases and cancer. Thus, targeted neutralization of their biological function has been in the center of many clinical trials aiming at treating these diseases. Recent studies from both of our groups show that several cytokines and chemokines display anti-inflammatory properties. In the current review, we focus on exploring this duality of function of chemokines and cytokines and its implications to autoinflammatory and inflammatory autoimmune diseases.

Diseases in which the immune system attacks and damages self-components are categorized as autoimmune or autoinflammatory diseases. In autoimmune diseases, cells of a specific arm of the immune system direct the pathogenesis. These diseases can be divided into those in which T cells are thought to direct the in-

Abbreviations: $^{-/-}$=deficient, DSS=dextran sulfate sodium, EAE=experimental autoimmune encephalomyelitis, FOXp3=forkhead box p3, GVHD=graft-versus-host disease, HCV=hepatitis C-induced vasculitis, IBD=inflammatory bowel disease, iTreg=induced regulatory T cell, MadCAM-1=mucosal addressin cell adhesion molecule 1, MS=multiple sclerosis, pM=picomolar, RA=rheumatoid arthritis, SDF-1=stromal cell-derived factor-1, T1DM=type I diabetes mellitus, Teff=effector T cell, Tfh=follicular Th, Tr1=type 1 regulatory T cell, Treg=regulatory T cell

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flammatory cascade, such as MS [5–7], T1DM [8], RA [9], or psoriasis [10–12], and into those in which antibodies are thought to be the key players, such as systemic lupus erythematosus, myasthenia gravis, Grave's disease, and others [13]. The interplay between antigen-specific $CD4^+$ T cells and B cells is essential for the pathogenesis of each type of these diseases. In T cell-mediated autoimmune diseases, the major damage to self-components is induced by effector $CD4^+$ Th1 cells that recruit monocytic cells [14–16], and by Th17 cells that recruit neutrophils and induce tissue-specific inflammation [17], as well as by $CD8^+$ T cells, which directly attack self-components [18, 19]. Hence, B cells are likely to contribute to the pathogenic process of these diseases in two ways: presenting autoantigens to Teffs and inducing ADCC and CDC at the site of inflammation [20]. This may explain why targeting B cells successfully suppresses these diseases [21–23]. As for antibody-directed autoimmune diseases, it is conceivable that in the vast majority of these diseases, antibody production is T-dependent.

In autoinflammatory diseases, such as the IL-1β-driven systemic juvenile idiopathic arthritis, the innate immune system is the key player in driving antiself-destructive immunity [24]. In all of these diseases, proinflammatory cytokines, such as TNF-α, IL-1β, and IL-12 and very recently, IL-17 and its receptor [10–12], have been valid targets for therapy of inflammatory autoimmunity and have met with significant success. Less successful were therapies aimed at targeting the function of several key chemokines, including CCL2 and its receptor (CCR2), which play a major role in directing the mobilization and homing of inflammatory macrophages to sites of inflammation [25], or IL-8 (CXCL8), a major neutrophil chemoattractant at sites of injury and inflammation [26]. The current review describes another face of several "inflammatory" mediators, as well as the additional activities of suppressor cytokines. We propose that the biological properties of these mediators vary depending on dose administered, stage of disease, and possible interplay with other cytokines.

CYTOKINES AND CHEMOKINES AS DRIVERS OF T CELL MIGRATION, POLARIZATION, AND BIOLOGICAL FUNCTION

Cytokines and chemokines are small cell-signaling proteins, whose receptors are expressed on immune cells. These factors play a critical role in immune cell differentiation, migration, and polarization into functional subtypes and in directing their biological functions. The surveillance of the body for foreign antigens is a critical function of the immune system. Lymphocytes migrate from the blood into tissues and secondary lymphoid organs and return to the blood via lymph vessels and the thoractic duct [27]. Naïve T lymphocytes traffic through the T cell areas of secondary lymphoid organs in search of antigen presented by DCs [28, 29]. These naïve cells express CD62 ligand and CCR7, which are required for their extravasation at the level of high endothelial venules [30–33]. Upon antigen recognition, specific T cells proliferate and in the presence of polarizing cytokines, differentiate into effector cells, which produce distinct patterns of cytokines and mediate various types of protective and pathological responses [34]. Differentiation of $CD4^+$ T cells into functionally distinct Th subsets is crucial for proper host defense and normal immunoregulation. Originally, $CD4^+$ T cells were viewed as having two possible fates: Th1 cells, which express T-bet and selectively produce IFN-γ, and Th2 cells, which express Gata3 and produce IL-4 [35, 36]. However, the Th1/Th2 paradigm failed to fully explain many phenomena of immunity and autoimmunity. More recently, cells that selectively produce IL-17 and the transcription factor retinoic acid receptor-related orphan receptor γt have been proposed to represent a distinct Th cell lineage, known as Th17 cells [37, 38]. Newer fates for Th cells continue to be identified, such as Th9 and Th22 cells, with classification based on production of their signature cytokines [39]. Tfh cells have been proposed recently as yet another lineage, with specialized function in helping B cells produce antibody responses [40, 41]. Extrinsic cytokine cues, namely IL-6 and IL-21, have been proposed to be the principal drivers of Tfh cell differentiation [42]. Tregs are another $CD4^+$ lineage with essential immunosuppressive functions that express the master transcription factor FOXp3 [43, 44] and comprise thymic-derived natural Tregs and peripheral iTregs.

Lymphocyte subsets express unique patterns of homing molecules, and the various types of vascular endothelium in different tissues express specific ligands, enabling migrating lymphocytes to be guided to their target tissue via site-specific pathways. A particular combination of trafficking molecules specifically targets antigen-experienced immune cells to different tissues expressing the respective ligands. After T cell differentiation, the LN homing receptors are down-regulated, while expression of tissue homing receptors is acquired, enabling the cells to migrate to peripheral sites. A specialized, stepwise cascade of events occurs, allowing circulating lymphocytes to gain access to the tissues [45–48]. Lymphocyte migration into tissues depends on regulation at each of these steps. In particular, lymphocyte migration is dependent on the ability of lymphocytes to interact with the endothelium of postcapillary venules through a multistep process governed by three prominent families of proteins: selectins, integrins, and chemokines. This process involves the capturing of lymphocytes by the endothelial cells, after which, lymphocytes "roll" over the endothelium and become "activated", a process usually mediated by several chemokines presented on the endothelial surface. This mechanism subsequently allows endothelial cells of the postcapillary venules to "arrest" lymphocytes on their surface and to guide them for extravasation into the target tissue [30, 49, 50].

The progress in understanding the mechanisms of T cell activation, T cell trafficking, migration, and homing, as well as progress toward development of mechanisms for the inactivation and modulation of T cells, has been translated into strategies for treatment of autoimmune diseases. The targets for selective immune intervention include modulation of antigen recognition, costimulation blockade, induction of regulatory cells, deviation to nonpathogenic or protective responses, neutralization of proinflammatory cytokines, induction or administration of anti-inflammatory cytokines, and modulation of leukocyte trafficking [51–54].

Accumulating lines of evidence show that inflammatory cytokines and chemokines participating in the immune response

can have stimulating and inhibitory effects on immune cells, depending on concentration, immune milieu, and interacting molecules (see Table 1). This review will discuss this dual behavior of these molecules.

CYTOKINES

Multiple cytokines can induce inhibitory and stimulatory effects on the immune system. This section will discuss the differential roles of TGF-β, IL-27, IL-10, IL-22, IL-2, and IFN-γ.

TGF-β

TGF-β plays a complex role in inflammation, T cell lineage commitment, antibody generation, immune suppression, and tolerance. In mammals, three isoforms of TGF-β have been identified: TGF-β1, TGF-β2, TGF-β3; among them, TGF-β1 is the isoform expressed predominantly by the immune system [55]. TGF-β mediates its biological effects through TGF-β type I and type II receptors, both of which are serine/threonine kinases. Upon TGF-β binding, a large receptor complex consisting of two TGF-βRI and two TGF-βRII molecules activates three receptor kinases, enabling them to phosphorylate downstream targets and to activate different signaling pathways [56]. TGF-β responses are mediated primarily via SMAD transcription factors.

TGF-β is known as a suppressor cytokine that is produced mainly by macrophages and by a special subtype of $CD4^+$ Tregs named Th3 cells, which take a major part in suppressing immunity within the gut [57]. Several studies demonstrated that TGF-β is critical, not only for the development and differentiation of FOXp3+ Tregs but also for the conversion of FOXp3− $CD4^+$ T cells into FOXp3+ iTregs [58, 59]. Moreover, together with IL-27, this cytokine polarizes IL-10^{high}FOXp3− Tregs [3].

Besides its role in immune suppression, it was discovered recently that TGF-β has a role in Th17 cell differentiation and function, revealing a proinflammatory effect of this cytokine. Development of Th17 cells is TGF-β-dependent as well, although coordinate signaling by IL-6, produced by DCs, activated by pathogens or IL-21, is necessary to induce the initial T cell commitment to Th17 cells [60, 61]. TGF-β and IL-6 together drive the differentiation of Th17 cells to a proinflammatory state with the usual physiological activity [56, 62, 63].

IL-27

IL-27 is a heterodimeric cytokine that consists of EBV-induced gene 3, an IL-12p40-related protein, and p28, an IL-12p35-related polypeptide [64]. IL-27 is an early product of activated APCs and drives rapid clonal expansion of naive but not memory $CD4^+$ T cells. It also synergizes with IL-12 to trigger IFN-γ production by naive $CD4^+$ T cells [64], functioning as a pro-Th1 inflammatory cytokine [65]. Accordingly, several groups, including ours, reported that its targeted neutralization during inflammatory autoimmunity suppresses Th1-directed diseases [66–68]. Is IL-27 indeed an inflammatory cytokine? Further exploration of its biological properties showed that it exhibits at least two different anti-inflammatory effects as well. Alone and together with TGF-β, it drives the polarization of IL-10-producing FOXp3–Tregs and therefore, could be used for suppression of inflammatory autoimmunity [3, 69]; in addition, IL-27 suppresses Th17 activities, via JAK1–STAT1 signaling, resulting in suppression of Th17-induced inflammatory autoimmunity [70–72]. This may explain, in part, why IL27R knockout mice display enhanced IL-17-producing T cell activity, resulting in development of a more severe form of EAE [71, 72]. These experiments contradict others showing that targeted neutralization of IL-27 supresses autoimmunity [66–68]. It is possible that IL-27 binds an additional receptor that mediates its proinflammatory activities, including directing Th1 polarization [64]. Further experiments using IL-$27^{-/-}$ mice are needed to elucidate this issue.

IL-10

IL-10 may be considered the most important anti-inflammatory cytokine in humans. It is produced by various cell populations, including macrophages and DCs, B cells [73], mast cells [74], and several subsets of T cells, including Th2 cells [75], Tregs [76], and Th17 [77] cells. IL-10 limits secretion of proinflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-12; it deactivates macrophages [78], inhibits secretion of Th1 cytokines, such as IL-2 and IFN-γ, and controls differentiation and proliferation of macrophages, T cells, and B cells [79–81]. By keeping proinflammatory events under control, it protects against excessive immune responses and tissue damage. FOXp3− Tregs release IL-10 as a major mechanism of their action, as do FOXp3+ Tregs, as one of their many immunosuppressive activities [76, 82]. In addition, myeloid cell-derived IL-10 maintains FOXp3 expression and suppressive function of Tregs in mice in various disease models [83], one of which is inflammatory autoimmunity within the gut [83]. It was shown recently that absence of this cytokine exclusively on FOXp3+ cells leads to loss of tolerance to gut inflammation [84]. In addition, IL-10 is involved directly in the selection and function of FOXp3− Tregs. Groux et al. [85] were the first to characterize IL-10^{high} FOXp3− Tregs, which are positively selected by this cytokine in an autocrine manner, to suppress inflammation. In a subsequent study, we showed that this process is a key event in infectious epitope spread of T cell tolerance during inflammatory autoimmunity [86].

IL-10 mediates most of its immunosuppressive activity indirectly, via effects on APCs, on which it down-regulates the expression of MHC [80] and B7 costimulatory molecules [87]. In addition, IL-10 limits proinflammatory cytokine and chemokine expression in APCs and can also directly affect CD4 T cells by limiting their activation, proliferation, and cytokine production [79, 88, 89].

Whereas these and other suppressor activities of IL-10 are largely accepted, there is less recognition of the pro-inflammatory potential of this cytokine. For instance, the first immune stimulatory function of IL-10 was observed in B cells. IL-10 up-regulates expression of MHC II on B cells [90]. It also serves as a survival factor for B cells and increases their antibody production [91]. IL-10 can also mediate immune stimulatory effects of T cells. Along with IL-2 and/or IL-4, it supports the growth and proliferation of mature and immature T cells [92]. It is therefore important to recognize that the net outcome of IL-10 activities is com-

plex and context-dependent, and in a given system, we may only see the net outcome of multiple effects.

IL-22

IL-22 belongs to a family of cytokines structurally related to IL-10, including IL-19, IL-20, IL-24, and IL-26. In contrast to IL-10, IL-22 has proinflammatory activities. IL-22 signals through a class II cytokine receptor composed of an IL-22-binding chain, IL-22RA1, and the IL-10RB subunit, which is shared with the IL-10R [93, 94]. Despite sharing a binding subunit with IL-10R, IL-22 has been mostly considered as a proinflammatory cytokine, particularly in skin and mucosal inflammation, where it is produced by Th17 cells in an autocrine response to IL-17 [95–97]. Recently, it has been shown that γδ T cells that accumulate at the skin produce IL-17 during skin inflammatory disease, promoting IL-22 activities [98]. These results have motivated two independent clinical trials aiming at targeting IL-17 or its receptor in attempting to treat psoriasis [10, 12]. Does IL-22 have anti-inflammatory properties related to autoimmune diseases? Besides T cells, IL-22 is largely produced by cells of the innate system, including NK cells [95, 99, 100]. A very recent study showed that the major function of IL-22 within the mucosal system is associated with keeping commensal bacteria contained in anatomical niches, preventing them from initiating an inflammatory process capable of inducing inflammatory autoimmunity within the gut [101]. This anti-inflammatory property of IL-22 should be remembered when considering anti-IL-22 or -IL-17 therapy for mucosal inflammation [102].

IL-2

IL-2 activates multiple immune-cell subsets, including T cells, NK cells, B cells, monocytes, macrophages, and neutrophils; it has therefore been used for therapy of various types of cancer, one of which is metastatic melanoma, in which it was used to support in vitro expansion of effector cells [103]. However, the use of IL-2 has been limited because of its toxicity at high doses and its limited efficacy when provided systemically. Nevertheless, among biological cancer therapeutics, IL-2 remains the preferred curative treatment for patients with metastatic renal-cell carcinoma [103]. In addition, it was recently shown that IL-2 is a powerful Th1 cytokine that induces a panel of chemokine/chemoattractant/retention receptor genes in Th subsets associated with skin and lung inflammation [104]. Does IL-2 also have anti-inflammatory properties? Four years ago, Tang et al. [105] used GFP–FOXp3 NOD mice to show that the primary function of IL-2 is the generation and survival of an essential $CD4^+$FOXp3+ Treg population (FOXp3+ Tregs), which functions to inhibit immune responses and prevent autoimmune diseases, such as T1DM in NOD mice. Grinberg-Bleyer et al. [106] subsequently extended this study showing the ability of low dose IL-2 to reverse later stages of this disease in NOD mice. Based on these results, very recently, two independent studies took this approach to human clinical trials in which low-dose IL-2 was applied to increase Tregs and treat HCV-induced vasculitis [107] and GVHD [108] with promising results. This is a classic example of how a single cytokine could be used, at different doses, to obtain opposing immunological effects. One of the major risks in using low-dose IL-2 for therapy of autoimmunity is its potential ability to promote the biological effector function of $CD8^+$ T cells and NK cells, which might aggravate the disease [19, 109].

IFN-γ

IFNs were characterized originally as a group of related molecules induced to mediate an immediate defensive response against viral infections [110, 111]. Type II IFN or IFN-γ was identified initially in the mid-1960s based on its antiviral activities [110] and is now known to play a much more important role as a general proinflammatory molecule, promoting essentially all aspects of the Th1 immune response, while suppressing Th2 and Th17 responses [112, 113].

When naive $CD4^+$ T cells (Th0, nonpolarized $CD4^+$ T cell) are activated in the presence of IFN-γ and IL-12, they differentiate into the Th1 subset [114]. IFN-γ production by $CD4^+$ T cells can control many aspects of inflammation and immunoregulation. For example, IFN-γ augments the antigen-processing and antigen-presenting ability of APCs, stimulates IgG2a production by B cells, induces the expression of cytokines and chemokines required for the recruitment of myeloid cells to the site of inflammation, and increases the expression of TLRs, NOS, and phagocyte oxidase by macrophages [115]. As a major effector cytokine of Th1 immunity, it is not surprising that IFN-γ autoamplifies Th1 responses and cross-inhibits differentiation and function of other Th subsets, including Th2 and Th17 cells. This regulation by IFN-γ represents a mechanism for maintaining Th1 lineage commitment and stabilizing Th phenotypes [114].

Despite its inflammatory activities, "paradoxical" findings indicated that IFN-γ could be protective in some models of autoimmune diseases such as EAE [116, 117] and asthma [118]. It was suggested that exacerbation of EAE in mice deficient in IFN-γ signaling correlates with reduced numbers and function of Tregs [119, 120], which serve to restrain overactivation of Teffs and maintain homeostasis. Moreover, adoptive transfer of IFN-γ-treated Tregs is sufficient to ameliorate EAE symptoms [119], supporting an essential role of IFN-γ in Treg development, at least in the EAE model. In addition, low doses of systemic IFN-γ were suggested to be involved in the negative regulation of lymphocyte homing to the LNs, resulting in down-regulation of the inflammatory response (see below) [118, 121].

Several studies suggested that IFN-γ function in inflammatory autoimmunity changes during different stages of autoimmune diseases. Targeting the function of IFN-γ during early EAE or inducing disease in IFN-$\gamma^{-/-}$ mice aggravates the disease, whereas administration of IFN-γ in the early phase reduces disease severity [122–124]. In a recent study, we showed that at very early stages of the development of EAE, $CD4^+$ T cells, expressing very high levels of IFN-γ, not only are not encephalitogenic but instead, mediate a protective effect [124]. Why so? At its early stages, the development of disease is largely dependent on Th17 $CD4^+$ T cells capable of initiating tissue-specific destruction [17]. IFN-γ suppresses Th17 to restrain autoimmunity [125, 126]. To further evaluate the hypothesis that IFN-γ^{high} Th1 cells function as Tregs at early stages of disease, we overexpressed IFN-γ in encephalitogenic T cells and demonstrated their ability to suppress EAE at its initial stages [124]. Moreover, the transition from a subclinical

form of disease to its pathological stage correlates over time with the apoptosis of such high IFN-γ-producing $CD4^+$ T cells, enabling a rapid increase in the Th17 population to initiate the disease process [124]. Interestingly, administration of IFN-γ, or IFN-γ-producing cells at later stages when the disease is mostly directed by Th1 cells, aggravates its manifestation [124], probably because at these stages, IFN-γ can no longer interfere with Th17 expansion and function. This exemplifies and provides a mechanistic basis explaining how the same cytokine at the very same concentration may induce diverse biological properties during an autoimmune disease in a time-dependent manner.

What is the relevance of these findings in human MS? A very recent study showed that based on immunological criteria, MS subjects could be divided into two major subgroups: those who are Th1-oriented in their $CD4^+$ T cell activities and those displaying predominant Th17 activities [127]. Patients who display a preferential Th1 response could potentially be better candidates for therapy with glatiramer acetate, also known as copolymer-1 (Copaxone), as this drug suppresses Th1 cells, whereas IFN-β-based therapies could be more effective in patients who display a Th17-oriented response [127]. Treating subjects who display a Th17-oriented response with copolymer-1 may worsen their Th17-based disease, as IFN-γ is a natural regulator of Th17.

Regulation of immature B cell homing to lymphoid and peripheral tissues by low-dose IFN-γ. Precursor B cells differentiate into immature B-lymphocytes after successful expression of a surface Ig receptor (IgM) [128, 129]. Newly generated, immature B cells are released from bone marrow and migrate to the spleen, where they differentiate into long-lived, mature B cells, prior to antigen encounter. Like other naïve lymphocytes, before their arrival in the spleen, immature B cells might recirculate to nonsplenic secondary lymphoid organs, which are specialized tissues for collecting antigens [130], or to sites of infection and inflammation. At these secondary lymphoid organs, which do not support final B cell maturation, premature antigen encounter would lead to the death of the immature B cells and elimination of effective clones as a result of to the negative-selection process. Therefore, before final maturation in the spleen, immature B cells are excluded from these sites.

Immature B cells regulate their homing in an autocrine manner by secreting low levels (~1000-fold less than the inflammatory levels) of IFN-γ, which engage the IFN-γR, leading to inhibition of cytoskeletal rearrangement; such rearrangement is required to promote integrin-mediated adhesion and migration of B cells [118, 131]. IFN-γ secretion by immature B cells is regulated by the MHC class I receptor, Ly49D. This activating receptor is expressed on peripheral immature B cells and recognizes MHC class I on peripheral tissues. The engagement of Ly49D by MHC class I molecules induces secretion of IFN-γ by immature B cells, thereby down-regulating their ability to home to the LNs or to sites of inflammation [132]. Ly49D activity is tightly regulated by the inhibitory receptor, Ly49G2. High levels of Ly49G2 have a dominant inhibitory effect on Ly49D expressed at low levels on immature bone marrow and mature B cells, resulting in inhibition of IFN-γ secretion. However, low levels of the inhibitory receptor Ly49G2, coexpressed with high levels of the activating receptor Ly49D, on the circulating immature B cells, enable the secretion of precisely regulated, low levels of IFN-γ. This expression pattern ensures the control of the migration of peripheral immature B cells, preventing their premature encounter with an antigen, while enabling entry to the LNs once they mature [133].

The cytokines IL-12 and IL-18 have been shown to stimulate IFN-γ production [134–136]. We showed that Ly49D stimulation leads to the elevated transcription and secretion of IL-18 and IL-12B (p40), resulting in augmented IFN-γ expression in immature B cells and autocrine control of their migration [137]. Ly49D-dependent secretion of IL-12 and IL-18 also induces IL-15 expression by immature B cells, which together, regulate IFN-γ production, inhibiting their ability to home to the LNs or to sites of inflammation [138].

Regulation of T cell migration by low-dose IFN-γ. Low doses of IFN-γ down-regulate integrin-mediated adhesion and migration of naïve T cells and exert an inhibitory effect on migration of effector Th2 cells in vitro. In addition, IFN-γ has a profound effect on the in vivo homing of naïve T cells to the LN, as well as the migration of the effector Th2 cell to sites of inflammation in an asthma model. Thus, in contrast to the proinflammatory effects of IFN-γ at relatively high concentrations, low-dose IFN-γ appears to exert global, suppressive effects on T cell trafficking and may have clinical application as an antiinflammatory agent [118]. It was suggested that the profound inhibition observed in the in vivo model might result from the combined effect of low levels of IFN-γ on various T cell populations. These low levels of IFN-γ down-regulate homing of naïve T cells to the LN and thereby, reduce the exposure of these T cells to antigen, and as a consequence, their activation is prevented. In addition, these low levels of IFN-γ dramatically inhibit homing of effector Th2 cells to sites of inflammation, where they exert their function. Thus, IFN-γ has a dual function in the asthma model, at the sensitization and effector stages, which results in a dramatic inhibition on the inflammatory response.

CHEMOKINES

Chemokines consist of a large family of small, structurally related chemoattractive cytokines (70–125 aa) [139]. Approximately 50 chemokines have been identified to date. These chemokines share conserved sequences and contain four conserved cysteines linked by disulfide bonds. Chemokines can be classified into four major subfamilies according to the configuration of these cysteine residues near the NH_2 terminus: CC, CXC, C, and CX_3C, where X stands for a separating amino acid [140]. Chemokines can be classified further according to their function and the regulation of their expression [141]. Some chemokines are important in the control of inflammatory processes, mainly via their chemotactic and proadhesive activities on numerous subsets of effector leukocytes [142], whereas others are involved in homeostatic trafficking of lymphocytes through lymphoid organs [47]. Inflammatory chemokines are produced mainly in nonlymphoid organs and primarily affect cells of the innate immune system, but they also affect different types of effector and memory lymphocytes and thus, help orchestrate innate and adaptive immune

responses [141]. Several cell types, including endothelial, epithelial, and stromal cells, as well as effector leukocytes, produce inflammatory chemokines [139, 143], and their expression is tightly regulated by inflammatory stimuli such as LPS, IL-1, TNF-α, and IFN-γ.

Homeostatic chemokines, on the other hand, are produced within primary and secondary lymphoid tissues and are involved in maintaining the lymphocyte and DC compartmentalization within these organs necessary for immune surveillance [144].

CCL2

CCL2 (MCP-1 in humans or JE in mice) is an important member of a large family of proinflammatory chemokines that plays a significant role in directing the influx of mononuclear leukocytes into sites of inflammation and tissue injury [145]. CCL2 is secreted by monocytes, memory T cells, macrophages, fibroblasts, endothelial cells, and mast cells and stimulates the movement of responsive leukocytes along a chemotactic gradient following binding to its cognate cell-surface receptor, CCR2 [146].

CCR2 serves as the receptor for CCL2 and is expressed on a variety of cell types of the immune system, including monocytes, activated T cells, NK cells, and DCs [147, 148]. Furthermore, it was shown that CCR2 is expressed on naïve and memory human tonsil B cells but not on germinal center B cells [147, 149]. The binding of CCL2 to the negatively charged extracellular loops of CCR2 is believed to initiate a signaling cascade, which plays a critical role in acute and chronic inflammatory processes [150]. Elevated expression of CCL2 and CCR2 has been observed in various diseases characterized by chronic inflammation and large numbers of infiltrating monocytes, including RA, MS, IBD, atherosclerosis, asthma, and uveitis [146, 150, 151]. In addition, $CCR2^{-/-}$ and $CCL2^{-/-}$ mice display reduced severity of EAE [152, 153], and targeted neutralization of CCL2 by DNA vaccines or antibody-based neutralization of soluble CCR2-Ig suppresses ongoing EAE [154, 155].

Although it is primarily an inflammatory chemoattractant for monocytes and effector T lymphocyte subsets at nanometer levels, CCL2 can exert, at pM levels three orders of magnitude lower than its chemoattractive concentration, global suppressive effects on lymphocyte trafficking directed by prototypic chemokine receptors such as CCR7 and CXCR4 [156]. CCR2 is transcribed in immature B cells, whereas its message is down-regulated dramatically at the mature stage. $CCR2^{-/-}$ cells exhibit up-regulation of chemokine-induced actin polymerization, migration, and homing to the LNs of immature B cells induced by other chemokines. The control of homing by CCR2 is mediated by its ligand CCL2, which is secreted by B cells and down-regulates the SDF-1 signaling cascade [157]. In addition, CCL2 (at pM levels) renders murine and human T cells defective in their ability to develop CCR7-triggered, LFA-1-mediated adhesion strengthening to endothelial ICAM-1 in vitro and in vivo. CCL2 also attenuates lymphocyte chemotaxis toward LN chemokines, such as CCL21 and CXCL12. Consequently, low-dose CCL2 inhibits lymphocyte homing to peripheral LNs but does not affect lymphocyte trafficking through the spleen. Impaired homing of lymphocytes to peripheral LNs results in attenuated progression of asthma and adjuvant-arthritis [156]. In addition, administration of pM levels of CCL2 markedly inhibits migration of T cells in amelioration of trinitrobenzene sulfonic acid and DSS colitis and inhibits development of colorectal cancer in an azoxymethane-DSS colitis model in mice. Thus, pM levels of CCL2 may be clinically beneficial as an anti-inflammatory agent in IBD [158] by exerting global suppressive effects on T cell trafficking and differentiation within peripheral LNs and may be clinically beneficial as an anti-inflammatory agent.

CXCL12

The CXC chemokine, CXCL12, which is also known as SDF-1 [159], was identified originally as a growth factor for murine pre-B cells [160]. It exists in two alternative splice variants: SDF-1α and SDF-1β. It is expressed constitutively by various cells and exhibits chemoattractive activity for monocytes, bone marrow 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 [161]. Furthermore, CXCL12 induces adhesion of T cells to ICAM-1 (CD54) [162] by up-regulating the binding activity of LFA-1 (CD11a/CD18) and modulates the adhesion of α4 integrins to VCAM-1, MadCAM-1, and fibronectin [163, 164]. In light of these findings, it is thought that CXCL12 plays an important role in the vascular trafficking and interstitital attraction of T and B cells to specific sites. 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 [165, 166]. Our findings highlight distinct nonchemoattractant functions of CXCL12 in shaping T cell differentiation and function, suggesting that this chemokine can exert strong, anti-inflammatory effects under various contexts [4].

In the healthy CNS, CXCL12 serves as a survival and migratory factor for neural and oligodendrocyte precursors that express the CXCR4 receptor [167]. The expression of CXCL12 within the CNS is up-regulated in the astrocytes of the MS brain, but its role in the regulation of this disease is not fully understood [168–170].

Our working hypothesis has been that targeted neutralization of CXCL12 during EAE would suppress the disease. In an attempt to neutralize CXCL12 at different time-points after active EAE was induced, contradictory effects were observed. As expected, administration of neutralizing antibodies to CXCL12, after induction but before the onset of disease, led to a delay of 2–3 days in the appearance of clinical signs (unpublished results). However, administration of these antibodies after disease onset resulted in severe exacerbation of the disease [4]. This suggests that CXCL12 plays a time-dependent, dual role in the regulation of inflammatory autoimmunity. The delayed onset of disease could be explained by the notion that at early stages of disease, the relative number of leukocytes that are recruited to the CNS is relatively low, and thus, CXCL12 enhances the activation of VLA-4 on these cells and contributes positively to its pathogenesis. This basic feature of CXCL12 is likely to be critical in preventing the development of progressive multifocal leucoencephalopathy that is increased significantly following anti-VLA-4 therapy [171]. In ongoing disease, when the inflammatory process within

TABLE 1. The Anti-inflammatory and Proinflammatory Features of Key Cytokines/Chemokines

Cytokine/ chemokine	Proinflammatory properties	Anti-inflammatory properties	References
TGF-β		Critical for the development and differentiation of FOXp3 + Tregs; directs the conversion of FOXp3 − to FOXp3 + induced Tregs; directly suppresses $CD4^+$ Treg functions; together with IL-27, polarizes Tr1	[3, 57–59]
	Together with IL-6, polarizes Th17 cells		[56, 62, 63]
IL-27		Together with TGF-β, polarizes Tr1; suppresses Th17	[3, 71, 72]
	Synergizes with IL-12 in polarizing effector Th1 cells		[64, 65]
IL-10		Directly and via polarizing Tr1, which produce IL-10, suppresses Teff functions and the inflammatory function of macrophages; IL-10 also has a key role in driving the suppressive properties of FOXp3 + Tregs	[78, 84, 85]
	Up-regulates expression of MHC II on B cells; serves as a survival factor for B cells and increases their antibody production		[90, 91]
IL-2		At low dose, serves as a critical survival/growth factor for FOXp3 + Tregs, and its low-dose administration suppresses autoimmunity and GVHD	[105–108]
	At high dose, promotes the activity of all Teffs		[103]
IFN-γ		Suppresses Th17 Teffs, thereby restraining Th17-directed autoimmunity; low doses of IFN-γ inhibit B and T cell homing to the LN and sites of inflammation; these doses suppress asthma	[118, 121, 124–126, 131, 137, 138]
	Simulates IgG2a production by B cells, drives Th1 polarization (together with IL-12), MHC II expression, and macrophage inflammatory activities		[113–115]
IL-22	Functions as a Th17 proinflammatory cytokine produced in an autocrine response to IL-17, particularly in association with skin and mucosal inflammation		[95–97]
		Involved in containing commensal bacteria in anatomical niches and thereby preventing them from initiating an inflammatory process capable of inducing inflammatory autoimmunity within the gut	[101]
CCL2	Directs the migration of CCR2 + monocytic cells to the site of inflammation to initiate the inflammatory process; thus, mice lacking CCR2 or CCL2 are EAE-resistant, and targeted neutralization of CCL2 is protective against this disease		[152–155]
		pM levels of CCL2 inhibit lymphocyte homing to peripheral LNs and sites of inflammation, resulting in attenuated progression of asthma, adjuvant-arthritis, and IBD	[156–158]
CXCL12		Selects IL-10, producing Tr1, which restrain autoimmunity; thus, its administration during an ongoing EAE episode suppresses progression	[4]
	Induces adhesion of T cells to ICAM-1, modulates the adhesion of α4 integrins to VCAM-1, MadCAM-1, or fibronectin		[162–164]

the CNS enters 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 [4]. Based on the above, we generated a CXC12–Ig fusion protein and explored its therapeutic capabilities in EAE, showing that administration of this fusion protein during an ongoing disease led to the selection of IL-10^{high}-producing Tr1 cells that home to the CNS to suppress the disease [4]. The implications of these results for human MS and other autoimmune diseases are that CXCL12 would probably be effective in suppressing these diseases if administered during an accelerating phase of relapsing-remitting diseases or during the progressive stage of ongoing chronic-progressive diseases. The effect of its administration during remissions in relapsing-remitting diseases has yet to be studied. This further exemplifies the importance of disease stage in applying new therapeutic approaches.

CONCLUSIONS

The results summarized here revise the traditional view of inflammatory cytokines and chemokines and the therapeutic implications of their activities (**Table 1**). The straightforward approach of targeting their biological activities and thereby suppressing inflammatory autoimmunity was demonstrated by successful therapy of RA using anti-TNF-α chimeric mAb ~18 years ago [172] and has since been extended to many diseases using mAb and soluble receptors that target various inflammatory mediators, including IL-1 [173], IL-6 [174], IL-12 [175], and very recently, IL-17 [10–12]. An alternative approach could include enhancing the activity of each of the two major types of $CD4^+$ Tregs—those that are FOXp3−, which mostly function by secreting IL-10 [124, 176, 177], or those that are FOXp3+ and operate by a large number of mechanisms [1, 2], including those that are IL-10-dependent [84]. Two recent studies extended this approach in humans using low-dose, IL-2-based therapy for treating HCV and GVHD [107, 108]. The current review extends this concept, showing that considering the dose of administration, the time-frame in the kinetics of disease, and possible combinations with other mediators, several key, proinflammatory mediators may be used to interfere successfully with the progression of these diseases.

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