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Junior Review Selection

The chemokine system: tuning and shaping by regulation of receptor expression and coupling in polarized responses

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Chemokines are a superfamily of small proteins with a crucial role in setup of immune and inflammatory reactions (1–4). At present, about 50 chemokines have been identified in humans. These molecules share a secondary structure with a flexible *N*-terminal segment followed by three antiparallel, β-sheets and a *C*-terminal α-helix, and according to the relative position of cysteine residues have been classified in four families (CXC, CC, C and CX3C).

CC chemokines are the most numerous and diverse family, including at least 25 ligands and 11 signaling receptors in humans (3–5) (Table 1). CC chemokines have been discovered following different pathways, ranging from biological and biochemical identification to direct cDNA cloning to, more recently, in silico cloning by gene-bank mining. For instance, in the case of CCL2/MCP-1, it had already been noted in the early 1970s that supernatants of activated blood mononuclear cells contained attractants active on monocytes and neutrophils. Subsequently, a chemotactic factor active on monocytes was identified in culture supernatants of mouse (6) and human (7) tumor lines and called tumor-derived chemotactic factor (TDCF) (7,8). TDCF was at the time rather unique in that it was active on monocytes but not on neutrophils (7) and had a low molecular weight (12 kDa) (7). Moreover, correlative evidence suggested its involvement in regulation of macrophage infiltration in murine and human tumors (7, 9). A molecule with similar cellular specificity and physicochemical properties was independently identified in the culture supernatant of smooth muscle cells (10). The JE gene has been identified as an immediate-early PDGF-inducible gene in fibroblasts (1l, 12). Thus, in the mid-1980s, this gene (JE) was in search for function and a monocyte-specific attractant was waiting for molecular definition. In 1989, CCL2/MCP-1 was successfully purified from supernatants of a human glioma (13), a human monocytic leukemia (14), and a human sarcoma (15–17): sequencing and molecular cloning revealed its relationship with the long-known JE gene (18–20).

Chemokines eponymous function is represented by induction of leukocyte migration, but these molecules also affect angiogenesis, collagen production and the proliferation of hematopoietic precursors. These

Abbreviations: DC, dendritic cells; HHV-8, human herpes virus 8; KS, Kaposi's sarcoma; LPS, lipopolysaccharide; TAM, tumor-associated macrophages; TDCF, tumor-derived chemotactic factor.

Table 1. Ligand and leukocyte specificities for human CC chemokine receptors

Systematic name	Chromosome	Main ligands	Main leukocytes
CCR1	3p21	CCL3, CCL3L1, CCL5, CCL7, CCL8, CCL14, CCL15, CCL16, CCL23	NK, Th1, Th2, iDC, Mo, Ba, Eo, Neu
CCR2	3p21	CCL2, CCL7, CCL8, CCL13, CCL16	NK, Th1, Th2, Mo, Ba
CCR3	3p21	CCL5, CCL7, CCL11, CCL13, CCL15, CCL24, CCL26	Th2, Ba, Eo
CCR4	3p22	CCL17, CCL22	Thy, NK, Th2, Tc2, Tm, Ba
CCR5	3p21	CCL3, CCL3L1, CCL4, CCL5, CCL8, CCL14	Thy, Th1, Tc1, iDC, Mo
CCR6	6q27	CCL20	Tm, B, iDC
CCR7	17q12–21	CCL19, CCL21	Thy, Tn, B, mDC
CCR8	3p22–p23	CCL1	Thy, Th2, Mo
CCR9	3p21.3–22	CCL25	Thy, Tm, B
CCR10	17q21.1–q21.3	CCL27, CCL28	Tm
CCR11	3p22	CCL19, CCL21, CCL25	T, iDC

Ba, basophils; Eo, eosinophils; iDC, immature dendritic cells; mDC, mature dendritic cells; Mo, monocyte-macrophages; Neu, neutrophils; NK, natural killer cells; Th, T helper cells; Thy, thymocytes; Tc, T cytotoxic; Tm, T memory cells; Tn, T naïve cells.
Ligand nomenclature according to reference (5).

biological activities are mediated by seven transmembrane domain, G-protein coupled receptors. At present, five receptors for CXC (CXCR1–5), ten receptors for CC (CCR1–10), one for C (XCR1) and one for CX3C (CX3CR1) chemokines have been identified (21).

CC chemokines act on a diverse spectrum of target cells (Table 1) mostly, but not exclusively, in the hematopoietic system. Nonhematopoietic elements, including epithelial cells, fibroblasts, and vascular elements have been shown to express receptors for and respond to CC chemokines, though the actual *in vivo* importance of these responses remains to be defined. The action of CC chemokines is regulated at the level of agonist production and processing as well as at the level of receptor expression and coupling. Therefore, an analysis of ligands must necessarily consider receptors.

In this review, we will summarize selected aspects of the pathophysiology of CC chemokines. In particular their role in the transition from innate to acquired immunity and in amplification of polarized responses will be discussed in more detail, and emphasis will be on selected molecules and pathologies used as a paradigm.

Regulation of production: constitutive *vs.* inducible CC chemokines

A useful classification of chemokines, more so of the most numerous CC family, distinguishes between molecules that are made in a tonic way (constitutive) and those that are made in response to diverse signals (inducible or inflammatory chemokines) (4, 5) (Fig. 1). Prototypic molecules of the constitutive CC chemokine realm are CCL14/HCC-1, present in normal plasma, and CCL18/PARC, constitutively expressed by dendritic cells. Prototypic molecules of the inducible CC chemokine realm include CCL2/MCP-1, CCL3/MIP-1αS, CCL4/MIP-1β and CCL5/RANTES. The general significance of constitutively expressed CC chemokines is to guide the normal traffic of leukocytes under normal conditions. For instance, CCL27/CTAK is probably important for regulation of traffic to cutaneous sites. CCL21/SLC and CCL19/ELC, expressed by lymphatic endothelium and by high endothelial cells, respectively, are important for guiding migration of lymphocytes and dendritic cells in lymphoid organs. The presence in normal plasma of substantial amounts of CCL14/HCC-1, CCL15/HCC-2 and CCL16/HCC-4, interacting with receptors widely expressed on peripheral blood leukocytes (CCR1, CCR5) has as yet unknown significance.

The general significance of inducible chemokines is to regulate recruitment of leukocytes on demand, in response to immunological, inflammatory and infectious signals. These two realms overlap in terms of molecules and pathology. As discussed below, constitutive expression of inducible chemokines is observed in neoplastic disorders. Moreover a number of molecules behave as both constitutive and inducible chemokines. For instance, CCL22/MDC was initially described as a chemokine constitutively expressed in certain cell types, most notably dendritic cells, and in certain lymphoid organs, in particular the thymus (22). Subsequent work, prompted by the recognition that this molecule attracted preferentially polarized type 2 T cells, has shown that CCL22/MDC is expressed in a regulated way. Expression of inducible chemokines is stimulated by signals that interact with diverse cellular receptors, including cells involved in innate immunity, pattern recognition receptors such as members of the Toll family, the T cell receptor, and costimulatory molecules for lymphocytes. As discussed below, chemokines are part of the circuit involved in generation and amplification of polarized type 1 and type 2 responses. It is therefore not surprising that master cytokines, which activate polarized responses differentially, regulate chemokine production (Fig. 2). For instance, the type 2 master cytokines, interleukin(IL)-4 and IL-13, induce production of agonists which interact with receptors that are preferentially expressed on polarized type 2 T cells, including CCL22/MDC and CCL17/TARC (agonists for CCR4), CCL11/eotaxin (agonist for CCR3)

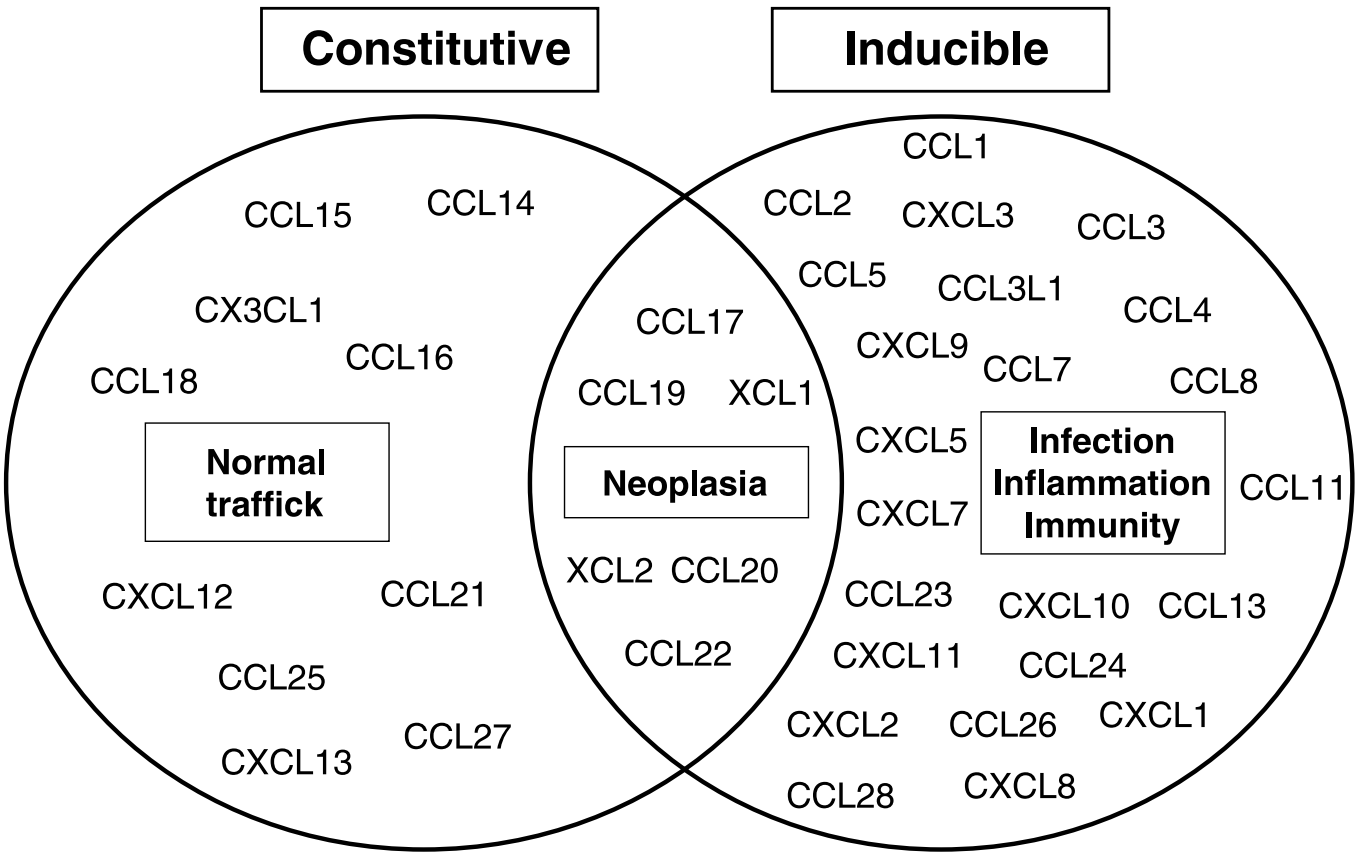

Figure 1. Functional classification of chemokines. According to regulation of their production, chemokines can be classified as constitutive chemokines, regulating normal leukocyte trafficking, or inducible chemokines, associated to inflammatory and immune responses.

and CCL1/I-309 (agonist for CCR8). Conversely, interferon(IFN)-γ as expected inhibits production of CCL22/MDC in different cell types and induces expression of CXCR3 agonists (3–5), as well as of CX3CL1/fractalkine. CX3CL1/fractalkine expression, the last identified chemokine involved in polarized responses (23), is induced by IFN-γ and tumor necrosis factor (TNF) and inhibited by IL-4 and IL-13. Hence, using CCL22/MDC as a paradigm, this chemokine is induced by generic stimuli representatives of interaction with microbial pathogen lipopolysaccharide (LPS) or immunocompetent cells (CD40L). In addition, the master cytokines IL-4/IL-13 and IFN-γ have reciprocal and divergent effects on production of this chemokine involved in the amplification of polarized type 2 responses (22).

Role of CD26/dipeptidyl-peptidase IV in chemokine processing

An additional level of regulation of chemokine function is represented by modifications at the *N*-terminus. Chemokines of both CXC and CC families are naturally post-translationally modified. For instance, two predominant forms of CXCL8/IL-8 were described to possess a different activity in neutrophil activation, and truncated forms of several CC chemokines were purified by cell supernatants and biological fluids (24). These truncations are due to the action of several proteases including attractin, plasmin, urokinase plasminogen activator and the CD26 dipeptidyl-peptidase IV (24). The role of CD26 in chemokine processing has recently focused interest greatly (24, 25). Originally identified as a marker of activated memory T lymphocytes (25, 26), CD26 is expressed in several cell types, including endothelial and epithelial cells. CD26 is a cell membrane-associated protein that is also found in a soluble form in seminal fluid, urine, and plasma. This protein exerts unique peptidase activity; it cleaves dipeptides from the *N*-terminus of proteins that have a Pro or Ala residue in the penultimate position. Several chemokines possess a proline residue in position 2. However, some of them, like CCL2/MCP-1, CCL7/MCP-3, CCL8/MCP-2 and CCL13/MCP-4 are protected from CD26 degradation by a pyroglutamate at the *N*-terminus that protects the protein by degradation. On the contrary, other chemokines can be effectively processed by the enzyme (27), and the biological output of this cleavage is unpredictable.

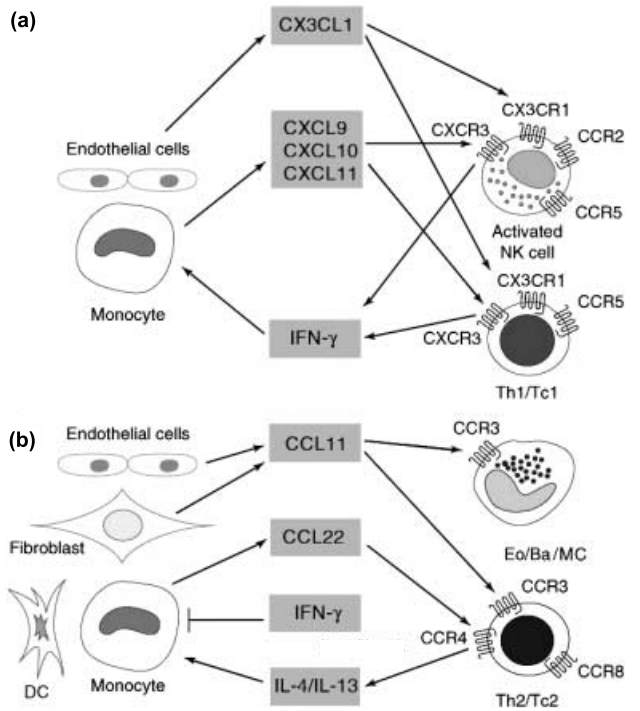

Figure 2. Chemokines in polarized type 1 and type 2 T-cell responses. During type 1 (a) and type 2 (b) immune responses, master cytokines regulate chemokine production by stromal and inflammatory cells. Chemokines then support selective recruitment of polarized T cells and specific type 1 and type 2 effector cells expressing distinct panels of chemokines receptors. Eo, eosinophils; Ba, basophils; DC, dendritic cells; IFN-γ, interferon-γ; MC, mast cells; NK, natural killer cells; Tc, cytotoxic T cells; Th, T helper cells.

Cleavage of CXCL6/GCP-2 does not modify its biological activity, but for most chemokines (CCL5/RANTES, CXCL12/SDF-1, CCL22/MDC, and CCL11/eotaxin) truncation by CD26 is accompanied by reduced, or somewhat altered, receptor binding and signaling. For all these chemokines a Pro or Ala—residue at the penultimate position is involved. An exception is CCL22/MDC, which is processed beyond this cleavage site. Very often, CD26-processed chemokines (e.g. CXCL12/SDF-1 and CCL11/eotaxin) not only loose their biological activity but also start to function as receptor antagonists, being able to inhibit the biological activity of intact proteins. Occasionally, the processed chemokine has a more complex biological behavior. CCL5/RANTES (3–68), functions as a receptor antagonist for CCR1 and CCR3, but shows an increased affinity for CCR5 (25). Similarly, CCL3L1/MIP-1αP (3–70) showed a 10-fold more efficiently and three-fold less efficiently than the full-length protein for the binding to CCR5 and CCR3, respectively, (28). The availability of new analytical techniques able to identify full-length proteins, as well as processed chemokines in biological fluids, will allow clarification of the role of CD26 in chemokine biology.

Regulation of receptor expression and coupling

CC chemokines are regulated not only by changing the levels of agonist production, but also by regulating expression of the appropriate receptors. One of the first observations made on this point was that monocytes exposed to bacterial lipopolysaccharide (LPS) showed a dramatic down-regulation of the receptor for CCL2/MCP-1, CCR2 (29, 30). This effect was associated with destabilization of the transcript, and was not dependent on induction of the agonist. Subsequent work has extended this observation to other proinflammatory signals, including TNF, IL-1 and IFN-γ as well as to other cell types such as dendritic cells (see below) and certain chemokine receptors on activated T and NK cells. These results suggest that down-regulation of certain inflammatory chemokine receptors (CCR2 most dramatically, CCR5, CCR1) may deliver a stop signal to recruited mononuclear phagocytes to focus their action at sites of infection and inflammation (Fig. 3). When these proinflammatory signals leak into the systemic circulation, by down-regulating CCR2 expression, they may provide a negative signal to inhibit excessive mononuclear phagocyte recruitment at sites of inflammation and tissue damage. Reciprocally, antiinflammatory signals such as glucocorticoid hormones and IL-10, were shown to increase expression of certain chemokine receptors, including CCR2 and CCR5 (29, 31, 32). Hence, pro- and antiinflammatory signals have reciprocal and divergent effects on expression of certain chemokine receptors in human mononuclear phagocytes, and this may serve as a strategy to fine tune the action of chemokines.

The results described so far refer to canonical cellular targets for CC chemokines, mononuclear phagocytes. It was also found that noncanonical cellular targets are rendered responsive to chemokines by microenvironmental signals. IFN-γ rendered neutrophils responsive to a

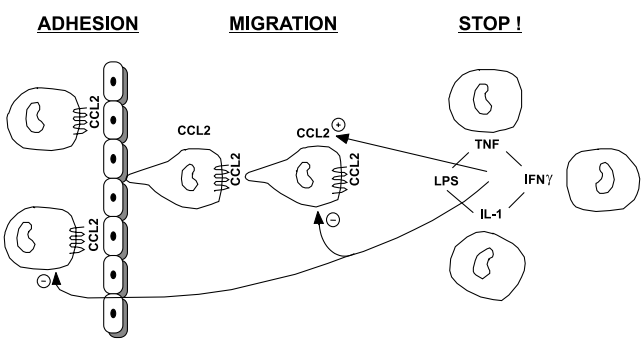

Figure 3. Down-regulation of inflammatory chemokine receptors by primary proinflammatory molecules as a stop signal. Primary proinflammatory molecules induce inflammatory chemokines to sustain cell recruitment from the blood stream. Once into the tissue, chemokine receptors are down-regulated to prevent chemokine degradation. Figure shows only CCR2 for simplicity.

series of CC chemokines, in particular CCR1 agonists (33). Mononuclear phagocytes express CXCR1 and CXCR2 but show little functional response. When cells are exposed to IL-4 and IL-13, increased receptor expression and coupling render these cells extremely sensitive to CXCL8/IL-8 and related CXC chemokines. Therefore, microenvironmental signals tune and shape the action of CC chemokines, by regulating receptor expression and coupling.

IL-10 tends to increase expression of certain inflammatory chemokine receptors, such as CCR2 and CCR5 (32). The significance of this observation may in fact relate to the effect of this cytokine when combined with primary proinflammatory signals (34). It was observed that IL-10 blocks down-regulation of inflammatory chemokine receptors induced by LPS alone or in combination with IFN-γ in monocytes and dendritic cells. However, monocytes and dendritic cells exposed to a combination of IL-10 and LPS, while showing high levels of CCR2 and CCR5, did not migrate in response to appropriate agonist and showed defective activation of signal transducing events. Inflammatory chemokine receptors in cells exposed to combination of LPS and IFN-γ retain the ability of binding and sequestering agonists. Evidence was obtained that chemokine scavenging may also occur *in vivo* in tissues where dendritic cells exposed to a combination of IL-10 and TNF are present. Hence, in an inflammatory environment dominated by IL-10, cells expressing inflammatory chemokine receptors are set in a chemokine scavenging, antiinflammatory mode. It was therefore suggested that these chemokine receptors act as functional decoy receptors for chemokines. More in general, it has been suggested that nonsignaling "silent" receptors act as decoy receptors for chemokines, including CC molecules (35).

Transition from innate to adaptive immunity: dendritic cells

Dendritic cells (DC) are potent antigen-presenting cells with a unique ability to capture antigens and to induce T- and B-cell responses (36). Chemokines, and especially CC chemokines regulate DC trafficking and localization. Immature myeloid DC express functional CCR1, CCR2, CCR5, that are likely responsible for their migration to the site of immune response. Blood plasmacytoid DC express a pattern of chemokine receptors similar to that of myeloid DC. However, most chemokine receptors of plasmacytoid DC, in particular those for inflammatory chemokines, are apparently not functional in circulating cells (37). Langerhans cells purified from skin or generated *in vitro* from $CD34^+$ precursors are characterized by the expression of CCR6, the receptor for CCL20/MIP-3α in addition to the receptors expressed by mono-DC (38). CCR6 interacts also with defensins that have agonist activity for DC (39). In the mouse, myeloid DC express CCR6 and localize to the subepithelial dome of Peyer's patches in response to CCL20/MIP-3α, which is strongly expressed by the overlying epithelium. CCR6 –/– mice have defective accumulation of DC in gut mucosal tissues and defective response to orally administered antigens. Thus, increasing evidence points to a distinct role of CCL20/MIP-3α and CCR6 in recruitment of DC towards the mucosal surfaces (40). Monocyte-DC also express two orphan receptors ChemR23 (41), and HCR (Otero and Sozzani, unpublished results). The role of these two receptors in DC biology is still unknown. Maturation of DC is associated with the inhibition of chemotactic response to inflammatory chemokines and the up-regulation of CCR7. CCL19/ELC and CCL21/SLC, the ligands of CCR7, are specifically expressed in T-cell rich areas of tonsils, spleen, and lymph nodes, where mature DC home, to become interdigitating DC (42, 43). The crucial role of CCR7 is clearly reflected in mice deficient for this receptor and the two ligands.

Overall these findings provide a model for DC-trafficking in which inflammatory chemokines, acting through CCR1 and CCR5 or CCR6, function as signals to localize DC precursors to peripheral tissues. After antigen uptake, immune or inflammatory stimuli induce DC maturation and loss of responsiveness to the locally produced inducible cytokines. This unresponsiveness may play a permissive role for DC to leave peripheral tissues. Meanwhile the slower up regulation of CCR7 prepares the cells to respond to CCL19/ELC and CCL21/SLC expressed in lymphoid organs.

DC also represent a source of chemokines *in vitro* and *in vivo*. *In vitro*, immature DC constitutively produce CCL22/MDC, CCL9/MIP-1γ and CCL18/PARC (43). Thymic DC selectively express CCL25/TECK, a CC chemokine active on thymocytes, macrophages, and DC. Production of chemokines by DC is strongly increased when these cells are induced to differentiate by proinflammatory stimuli (e.g. LPS, TNF) or engagement of CD40. *In vitro*, mature DC produce conspicuous amounts of CCL2/MCP-1, CCL3/MIP-1αS, CCL5/RANTES, and CXCL8/IL-8 (44, 45). Mature DC also produce very high concentrations of CCL22/MDC and CCL17/TARC (45). *In vivo*, CCL22/MDC was detected by *in situ* hybridization in mature DC and the protein is strongly produced by $CD83^+$ cells in the skin of atopic dermatitis patients (45). CX3CL1/fractalkine and CXCL16, two chemokines present in both a membrane anchored and a soluble forms are produced by mature DC (46, 47). CXCL13/BLC is produced by follicular DC and germinal center DC and it is likely to play a role in interaction of DC with B and T lymphocytes (48). DC-derived chemokines are believed to contribute to the recruitment of precursor cells and immature DC at peripheral sites of inflammation (42, 49, 50). Furthermore, within the lymph nodes, chemokines may also play a role in T- and B-cell localization and in interaction between DC and T cells (36, 42).

Polarized responses

Homeostatic CC chemokines play a role in regulating the normal traffic of lymphocytes in lymphoid organs (29, 51). The CCR7 receptor is expressed on mature DC (see above), naive T cells and a subset of memory T cells (central memory T cells) (52). CCR7 is recognized by two ligands, CCL21/SLC (present in two forms in the mouse differing by one amino acid, Ser–Leu, at position 65) and CCL19/ELC. CCL21/SLC is expressed on lymphatic endothelial cells whereas CCL19/ELC is expressed by various cell types, including high endothelial venules. Analysis of the mouse mutant *plt* and of CCR7 –/– mouse has given results consistent with a nonredundant role of this CC chemokine receptor in regulating traffic of lymphoid cells and DC in lymphoid organs (53, 54). As discussed above, gene targeting has also revealed unequivocally a role for CCR6 in the regulation of DC traffic in the mucosal compartment. CCL17/TARC and CCL27/CTAK are important for the traffic of skin homing T lymphocytes (55, 56). CCL17/TARC recruits CLA^+ T cells, which are the predominant type present in the skin. CCL17/TARC is expressed on skin endothelial cells. Moreover, CCL27/CTAK, a chemoattractant expressed widely in the skin, being produced by keratinocytes, is active on CLA^+ T cells. Therefore, this tissue specific CC chemokine can direct migration of the CLA^+ T cell subset to this anatomical site.

There is compelling evidence that a set of chemokines are involved in polarized Th1/Th2 responses (4, 29, 51, 57, 58). Polarized type 1 and type 2 T cells express differential chemokine receptors. Typically, the CC chemokine receptors CCR3, CCR4 and CCR8 have been associated with a type 2 phenotype, whereas functional CXCR3 and CCR5 are preferentially expressed on polarized type 1 T cells (Fig. 2). CCR3 ligands also attract eosinophils and basophils, crucial for polarized type 2 responses. Differentially expressed chemokine receptors are not markers for polarized T cells, in that there is no absolute association between chemokine receptor expression and cytokine repertoire of polarized T cell populations. In contrast, the seven transmembrane domain receptor CRTH2 has been shown to be more strictly associated with the type 2 phenotype than are chemokine receptors (59). For instance, CCR4, while expressed at much higher levels in polarized type 2 cells, it is induced in type 1 cells following activation, and it is also expressed in nonpolarized T cell populations. Moreover, CCR4 is thought to be involved in homing to the skin. As discussed above and described in Fig. 2, consistent with the role of polarized T cell responses, agonist for receptors expressed differentially in polarized T cells are differentially induced/inhibited by IL-4, IL-13 and IFN-γ. For instance, the CCR4 agonist CCL17/TARC and CCL22/MDC are induced by IL-4 and IL-13 and inhibited by IFN-γ. A CCR4 –/– mouse has been generated, but no evidence for alteration in polarized responses was observed. However, antibody against CCL22/MDC blocked recruitment of T cells in a classic model of airway hyperreactivity. No abnormalities in airway hyperreactivity were observed in CCR3 –/– mice. In contrast, CCR8 –/– mice were protected against higher airway hyperreactivity. Recent careful analysis of chemokine receptors expressed in T cells in asthma has yielded results consistent with a role of this molecule in allergic inflammation (60). It was found that in allergic asthma CCR3 is rarely expressed in T cells, unlike CCR4 and CCR8. Collectively, these results suggest that chemokines play a key role in the induction and amplification of polarized responses and may represent an important target for therapeutic intervention.

In addition to playing a role in the effector phase of polarized responses, chemokines may be important in their induction. Analysis of responses to a parasite component (STAG) has suggested a key role for CCR5 expressed on dendritic cells not only in their migration but also in the induction of IL-12 (61). Studies in CCL2/MCP-1 –/– mice have indicated that this chemokine may be important for the induction and expression of polarized type 2 responses. Interestingly, data in CCR2 –/– mice have pointed to the opposite direction as far as polarized responses are concerned (33, 62). In contrast, consistent results between gene targeting of the agonist and of the receptor have been obtained in terms, for instance, of susceptibility to atherosclerosis. Indeed, in a redundant and promiscuous system in which several agonists interact with one receptor, inactivation of one of them is not necessarily equivalent to receptor blockade. Receptor inactivation may block one pathway that scavenges the agonists. Therefore, in the MCP system, this may result in increased concentration of members of the family such as CCL7/MCP-3 which in addition to interacting with CCR2 interacts with CCR3, a receptor expressed on a subset of polarized type 2 cells, as well as on eosinophils and basophils. This may explain the apparent discrepancy in terms of influence on polarized responses obtained between studies in agonist (CCL2/MCP-1) and receptor (CCR2) gene targeted mice.

Chemokines in tumors

Most tumors, if not all, produce chemokines (63). Compelling evidence in murine models and in human tumors suggests that CCL2/MCP-1 and related CC chemokines such as CCL5/RANTES are major determinants of macrophage and lymphocyte infiltration in melanoma, in carcinoma of the ovary, breast and cervix, and in sarcomas and gliomas (64–69). In Hodgkin's disease the malignant Reed–Sternberg cells express the type 2 T-cell attracting chemokines CCL22/MDC and CCL17/TARC (70, 71) and the chemokine CCL11 eotaxin, produced by stromal elements, correlates with eosinophil infiltration

(72, 73). Chemokine production by tumor cells may reflect constitutive gene expression or activation by autocrine/paracrine loops, as shown for fas/fasL in brain tumors and IL-6 in cervical neoplasia (74, 75). Chemokine receptors are expressed both by infiltrating leukocytes and by tumor cells. The former may lose receptor expression once they are exposed to cytokines in the tumor microenvironment, as shown for CCR2 on tumor-associated macrophages (TAMs) in ovarian cancer (76). In the murine and human tumors studied, TAMs have a skewed type 2 phenotype. They do not produce IL-12 and NFκB activation is lacking. TAMs spontaneously release conspicuous amounts of IL-10 and TGF-β (77–79). Autocrine production of IL-10 may be responsible for the phenotype of TAMs (77). The mechanisms responsible for the polarization of TAM to a type 2 phenotype are a matter of speculation. A Th2/Tc2 dominated T-cell response *in situ*, with production of IL-4 and IL-13 (70, 80, 81), would drive a type 2 inflammatory response. Some chemokines, including CCL2/MCP-1, induce IL-10 in macrophages. Moreover, data in knockout mice suggest that CCL2/MCP-1 polarizes immunity in a type 2 direction (62). Chronic exposure to high chemokine concentrations in the tumor microenvironment may set in motion a chemokine-centered vicious circuit, leading to skewing towards a type 2 inflammatory response (77).

Some viruses encode chemokines, chemokine inhibitors, and chemokine receptors. Of particular interest is human herpes virus 8 (HHV-8) which is involved in the pathogenesis of Kaposi's sarcoma, KS, and body-cavity lymphoma. In addition to encoding a constitutively active chemokine receptor, which acts as a dominant oncogene (82, 83), HHV-8 contains three chemokines, called vMIP-I, vMIP-II and vMIP-III, that are selective attractants of polarized type 2 T cells and interact with chemokine receptors (CCR3, CCR4, CCR8) expressed on this population (80, 84–86). T cells infiltrating KS, which are predominantly $CD8^+$, have a skewed type 2 phenotype (80). HHV-8 encoded chemokines might thus be a strategy to subvert immunity, by activating type 2 responses and diverting effective type 1 defence mechanisms (summarized in Fig. 2).

In terms of inflammatory reactions, neoplastic disorders constitute an apparent paradox. As discussed above, many tumors, if not all, produce inflammatory cytokines, chemokines and are infiltrated by leukocytes. However, neoplastic disorders are associated with a defective capacity to mount inflammatory reactions at sites other than the tumor, and circulating monocytes from cancer patients are defective in their capacity to respond to chemoattractants (63, 87). Various factors originating in the tumor microenvironment may contribute to the systemic antiinflammation associated with cancer. Chemokines leaking into the systemic circulation are likely to desensitize circulating leukocytes (88); elevated levels of TNF receptors and the type 2 decoy IL-1 receptor may buffer inflammatory cytokines; and tumors also produce antiinflammatory cytokines such as IL-10 or TGF-β (77–79). On this basis, a defective capacity to mount a systemic inflammatory response in cancer patients coexists with continuous leukocyte recruitment at the tumor site. CC chemokines induce production of proteases, such as matrix metalloproteases and urokinase-type plasminogen activator in tumor cells and macrophages (see below). These enzymes are important for invasion and it has been suggested that monocytes infiltrating the tumor tissue provide cancer cells with a ready-made path for invasion (countercurrent invasion theory) (89). As described above, tumor cells may also express chemokine receptors. Appropriate chemokine agonists induce migration or proliferation of tumor cells, raising the interesting possibility that tumor cells may use chemokine gradients to spread around the body (90–95). It has recently been found that breast carcinoma cells and melanoma cells express CCR7 and CXCR4 and these may be important for metastasis (92). Receptors such as CCR7, essential for lymphocyte and dendritic cell homing to lymph nodes, could play a similar role for lymphatic dissemination of certain carcinomas. Direct evidence for chemokines guiding the secondary localization of cancer has been obtained (90, 92, 96, 97).

The finding of constitutive production of chemokines in tumors is consistent with the view that recruited leukocytes, macrophages in particular, provide a direct or indirect mechanism for tumor promotion (63). The protumor function of chemokines is strictly related to the amounts produced. It has been shown that low level of CCL2/MCP-1 production renders nontumorigenic melanoma cells capable of forming progressing tumor lesions (98). In contrast, forced expression of high levels of CCL2/MCP-1 and other CC chemokines has caused tumor inhibition (3–5, 99). In this context, expression of CC chemokines capable to attract dendritic cells such as CCL7/MCP-3 and CCL21/SLC, was associated with dendritic cell recruitment, activation of specific immunity, and tumor regression (100–103).

The transcription program activated by CC chemokines

Various indications in the literature suggest that chemokines have a role in the development of different biological responses that goes beyond cell recruitment. For example, CXCR2 agonists support tumor growth through induction of growth factors in malignant cells (104), as also demonstrated for the related constitutively active GPCR ORF74 receptor encoded by HHV-8 (105). Chemokines have also been shown to play a direct role in definition of the cytokine milieu during both inflammatory (106) and immune responses (107, 108). Thus, chemokines not only support differential leukocyte recruitment, but also directly affect target cell functions. Gene chip-based gene expression profile analysis in

Table 2. Comparison of transcriptional profile induced by CCL5 and LPS in monocytes

	LPS Induced	LPS not affected	LPS Repressed
CCL5 Induced	10 (0.2%)	16 (0.3%)	16 (0.3%)
CCL5 not affected	84 (1.7%)	1385 (27.7%)	10 (0.2%)
CCL5 Repressed	0 (0%)	0 (0%)	0 (0%)

Monocytes were stimulated in nonadherent conditions for 2 h with 100 ng/ml lipopolysaccharide (LPS) or 300 ng/ml CCL5 and transcriptional profile was analyzed using Affymetrix HuGene FL arrays (Affymetrix, Santa Clara, CA, USA), interrogating 5200 human genes. Percentages indicate the fraction of cell transcriptome regulated.

chemokine-activated monocytes revealed that CC chemokines induce specific transcriptional programs in target cells (Table 2, 109), demonstrating that chemokines effects on target cells include induction of transcriptional events. CCL5/RANTES induced a significant fraction of transcripts, which included inflammatory cytokines, indicating an active role for chemokines in the organization of the inflammatory reaction, and chemokines and their receptors, as previously reported (110), suggesting an amplification and diversification cascade for leukocyte recruitment. The CCL5/RANTES-activated transcriptional program also supports cell migration and tissue penetration, as demonstrated by induction of proteases and adhesion molecules acting on subendothelial structures. On the contrary, consistent gene suppression was not observed. Comparison with the classical inflammatory mediator LPS demonstrated that chemokines activate a specific transcriptional program partially overlapped but clearly distinct from that induced by LPS.

Concluding remarks

Chemokines are a complex system of molecules that affect a variety of hematopoietic and nonhematopoietic cell types. At least some of these molecules and their cognate receptors are interesting targets for pharmacological intervention (111). Validation as pharmacological targets includes gene targeting (e.g. CCL2/CCR2; CCR1; CCR7; CCR8), usage of antibodies or antagonists (e.g. antiCCL22/MDC; MetRANTES), and expression in human pathology (e.g. CCR4 and CCR8 in asthma), as discussed in this review. Chemokine receptor antagonists have been developed (e.g. CCR5, CCR1) and are at various stages of preclinical or clinical development. The development of efficacious chemokine antagonists remains a "holy grail" for the general field of cytokine pharmacology.

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