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Chemokines and chemokine receptors: an overview

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1. ABSTRACT

Chemokines are chemotactic cytokines orchestrating leukocyte recruitment in physiological and pathological conditions. This complex system includes 42 molecules and 19 receptors and is subjected to different levels of regulation, including ligand production, post-translational modifications and degradation, as well as receptor expression and signaling activity. Here we analyze the chemokine system, with particular attention to available information on clinical situations in which chemokines or their receptors might assume diagnostic value.

2. INTRODUCTION

Chemotactic cytokines, or *chemo-kines*, are a large subfamily of cytokines that coordinate leukocyte recruitment and activation, two crucial elements in the pathogenesis of several immuno-mediated human diseases. Chemokines have been recognized in the last few years as important mediators in the pathogenesis of many human diseases and have assumed growing relevance in clinical pathology as markers of disease onset, progression, and remission.

3. THE CHEMOKINE SYSTEM

3.1. The repertoire of chemokines and chemokine receptors

Since the description of the first chemokine in 1977, over 40 related molecules have been discovered in humans and chemokines have been recognized as a family of functionally related small secreted molecules named "chemo-kine" because of leukocyte chemoattractant and cytokine-like activities (1-3). Although most members share functional properties, membership is governed exclusively by structural criteria. Chemokines are composed of single polypeptide chains 70-100 aminoacid residues in length, with 20-95% sequence identity to each other including conserved cysteine residues that have also been used for subfamily definition and nomenclature. According to cysteines number and spacing, four chemokine subfamilies have been defined (Figure 1). The largest group of chemokines has the first two of total four cysteines in adjacent position (CC chemokines). These molecules represent the SCYb genes product and are in large part clustered on chromosome 17q11.2 (4). The major cellular target for this cluster of molecules is represented by monocytes, while other CC chemokines coded on different chromosomal loci are active on different cell types. The other large group of chemokines has the first two of the four total cysteines separated by an intervening aminoacid (CXC chemokines). These molecules represent the SCYa genes product and are in large part clustered either on chromosome 4q12-q13 or on chromosome 4q21.21. The major cellular target of the first cluster of molecules is represented by neutrophils, while the second cluster is mainly active on lymphocytes. Both CC and CXC subfamilies include many members, and members of one subfamily resemble each other more than with members of the other subfamily. More recently a small number of chemokines that does not fit this structural profile have been described. A third subfamily includes two molecules with only two cysteine residues (C chemokines). These two related chemokines represent the protein product of the SCYc genes located on chromosome 1q23 and are selectively active on T lymphocytes. Only one molecule with three intervening aminoacid in-between the first two cysteine residues have been described so far. This molecule is the protein product of the SCYd gene located on 16q13 and may represent the first representative of a putative fourth chemokine subfamily indicated as CX3C chemokines. Members of this new family may also present a quite new structural organization, since this chemokine has a transmembrane domain which allows it to be tethered to the cell surface. Chemokine nomenclature is based both on structural features related to cysteine residues position and receptor usage (5). CC chemokines, being CCR ligands, are indicated as CCL followed by a number provided by the corresponding SCYb coding gene. Similarly, CXC chemokines are named CXCL, C chemokines XCL and CX3C chemokines as CX3CL. Basically, chemokines are now identified by a name providing information on the respective structural subfamily and the type of receptor they engage, followed by a consecutive number provided by and referring to the respective coding gene.

Receptor usage is the major functional correlate of chemokine subclassification (6). The 19 known receptors typically bind multiple chemokines in a subclass-restricted manner (Figure 1). Thus, their names include the chemokine subclass specificity followed by a number (CCR1-10, CXCR1-6, XCR1, CX3CR1). Chemokine receptors define a distinct subfamily in the rhodopsin-like G protein-coupled receptors, being single polypeptide chains spanning 7 times the membrane, with an acidic N-terminal extracellular domain and a serine/threonine-rich intracellular C-terminal domain. Two disulfide bonds in-between the N-terminal domain and the second extracellular loop and the first and third extracellular loops are normally required for the definition of the molecular structure. As with ligands, also for receptors 20-85% aminoacid identity to each other is observed, focused in particular in transmembranes and intracellular domains. The significant identity score and some structural aspects indicate that both ligand and receptor families arose from common ancestors by repetitive gene duplication.

3.2. Sources and targets of chemokines

Although virtually all chemokines act as intercellular signals, their sources, targets and regulation vary widely, presumably to match the complexity of the hematopoietic system and in part to provide flexibility and specificity in leukocyte trafficking and other functions (7). Most chemokines are produced by many cell types, but some are produced by as few as one. Some can be produced constitutively, others must be induced, and some are produced both constitutively and inductively, depending on the cell type. Inducible chemokines vary according to inducing agent specificity. Many are up-regulated by pro-inflammatory cytokines, such as tumor necrosis factor (TNF) alpha and IL-1; others are up-regulated specifically by interferon (IFN)-gamma, and most are down-regulated by the anti-inflammatory cytokine IL-10 (8). An exception is represented by CCL16/HCC4, which is up-regulated by IL-10 and could act as an "off" signal during the inflammatory response (9). Most inducible chemokines are regulated at the transcriptional level, but some are stored for immediate release, as in the case of CXCL4/PF-4 in platelet alpha granules (10).

Both narrow- and broad-spectrum chemokines exist. The spectra overlap widely, and together span all leukocytes. Neutrophil-targeted chemokines are found mainly in the CXC subfamily, whereas monocyte/macrophages, eosinophils, and basophils are mainly attracted by CC chemokines. Another element of structure that correlates strongly with target specificity is the presence of the tripeptide glu-leu-arg motif (ELR in short) near the N terminus of neutrophil-targeted CXC chemokines (see later). CXCL13/BCA-1 is a CXC chemokine specific for B lymphocytes (11). Multiple broad-spectrum CC chemokines are known, whereas CXC chemokines tend to be more restricted. Both CC and CXC subfamilies also contain T lymphocyte-specific members, and specific CC receptors mark Th1 (CXCR3, CCR5) versus Th2 (CCR3, CCR4) subsets (12).

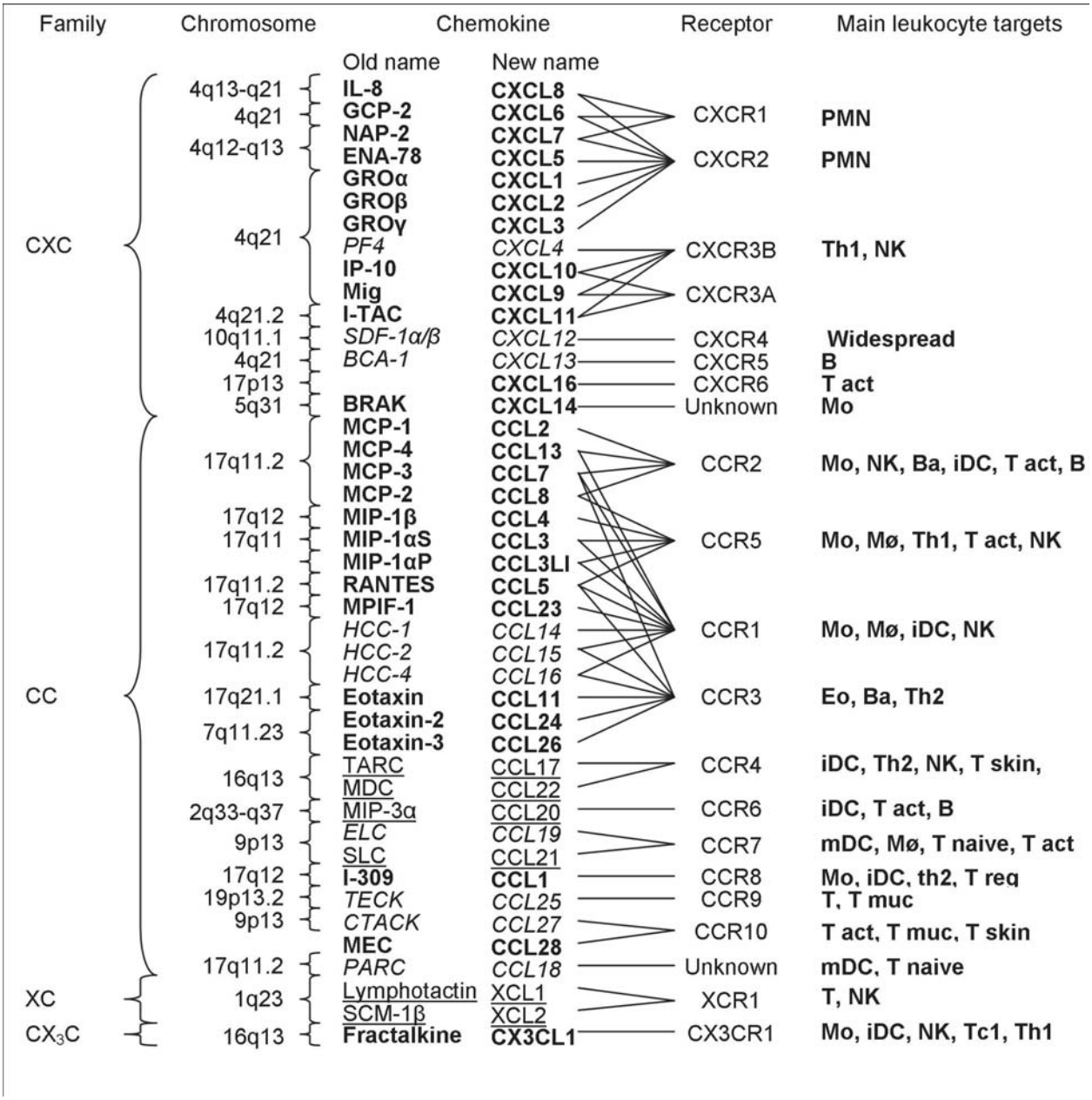

Figure 1. The chemokine system: an overview. Chemokines (family, chromosome location, and old and new nomenclature), their receptors, and predominant receptor repertoires in different leukocyte populations are listed. Names in bold identify inflammatory chemokines, names in italics homeostatic chemokines, underlined names refer to molecules belonging to both realms. Chemokine acronyms are as follows: BCA, B cell activating chemokine; BRAK, breast and kidney chemokine; CTACK, cutaneous T-cell attracting chemokine; ELC, Epstein–Barr virus-induced receptor ligand chemokine; ENA-78, epithelial cell-derived neutrophils-activating factor (78 amino acids); GCP, granulocyte chemoattractant protein; GRO, growth-related oncogene; HCC, hemofiltrate CC chemokine; IP, IFN-inducible protein; I-TAC, IFN-inducible T-cell a chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage derived chemokine; Mig, monokine induced by gamma interferon; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; NAP, neutrophil-activating protein; PARC, pulmonary and activation-regulated chemokine; RANTES, regulated upon activation normal T cell-expressed and secreted; SCM, single C motif; SDF, stromal cell-derived factor; SLC, secondary lymphoid tissue chemokine; TARC, thymus and activation-related chemokine; TECK, thymus expressed chemokine. Leukocyte acronyms are as follows: PMN, neutrophils; Eo, eosinophils; Ba, basophils; MC, mast cells; Mo, monocytes; Mø, macrophages; iDCs, immature dendritic cells; mDCs, mature DCs; T naive, naive T cells; T act, activated T cells; T skin, skin-homing T cells; T muc, mucosal-homing T cells; T reg, regulatory T cells.

There is considerable variability in the extent of chemokine redundancy (7). For example, CXCL12/SDF-1 and CXCR4 are monogamous signaling unit on many cell types excluding mature B cells, and CXCL13/BCA-1 and CXCR5 are a monogamous signaling unit restricted to mature B cells; but CCL5/RANTES signals promiscuously through CCR1, CCR3 and CCR5, which are differentially expressed on basophils, eosinophils, monocytes, and T cells. Different receptors for the same chemokine can be co-expressed at similar levels on the same cell type. One model is that receptors are used sequentially in successive gradients of chemoattractants, balancing desensitizating and activating signals.

Chemokine receptor regulation also varies. Intuitively, one would imagine that a trafficking system would include inducible signals and constitutively expressed receptors, and this is the case for most chemokine receptors, although requirements for up- and down-regulation vary. However, several receptors are detected exclusively in specific cell states, e.g. CXCR3 on activated T cells (13).

3.3. Chemokines and the multistep model of leukocyte trafficking

Leukocyte contact with endothelium may be transient, reversible and activation-independent. If sustained, cells roll across the endothelial surface by chemokine-independent interactions of selectins with counteradhesins. At inflamed sites, the leukocyte enters a second phase involving chemokine-dependent β2 integrin activation and high-affinity binding to endothelial cell receptors (14). Evidence is accumulating that leukocytes interact with chemokines that are immobilized on surface by proteoglycans (15). This would prevent washout by blood flow and would promote gradient formation on endothelial glicocalix and extracellular matrix, which the leukocyte follows towards the focus of inflammation. The simultaneous action of chemokines and integrins is needed for full activation of leucocytes, in synergy with primary cytokines. This process enhances phagocytosis, superoxide production, granule release and bactericidal activity *in vitro*. *In vivo*, however, chemokines may primarily induce leukocyte accumulation without activation, as suggested by overexpression experiments in transgenic mice (3, 16).

4. CHEMOKINE SYSTEM REGULATION

4.1. Regulation at the level of chemokine production

Depending on their regulation and production, chemokines can be distinguished in "homeostatic" chemokines, which control leukocyte homing and lymphocyte recirculation in normal conditions, and "inflammatory" chemokines, produced in response to inflammatory stimuli, like TNF or IFN, in order to recruit leukocytes during inflammation (Figure 2A) (7). Chemokines play a role in the polarization of type I and type II immune responses, supporting selective recruitment of polarized T cells and specific type I and type II effector cells expressing distinct panels of chemokine receptors (12). Chemokines can also be considered molecular markers of different types of inflammatory macrophages, in that a different panel of chemokines characterizes M1 and M2 macrophages, involved respectively in type I and II immune response (8).

4.2. Regulation at the level of chemokine processing

Post translational modifications naturally occur for both CXC and CC chemokines and strongly affect their biological activities (17). Several proteases, including metalloproteinases, thrombin, and plasmin, act on different chemokines leading to the production of distinct variants that differ by the extension of the amino-terminal or, less frequently, carboxi-terminal region. Several evidences indicate that extracellular processing of chemokines by proteases is an important way to regulate the activity and the function of the chemokine system in different physiological and pathological conditions. As an example, CCL8 (6-76), a truncated variant of CCL8/MCP-2, is biologically inactive and acts as a receptor antagonist by blocking the chemotaxis induced by CCL5/RANTES and CCL2/MCP-1. Similarly, the Th1-restricted membrane protease CD26 cleaves the CCR4-agonist CCL22/MDC, generating two truncated forms of the chemokine, CCL22 (3-69) and CCL22 (5-69), with reduced capacity to interact with their cognate receptor CCR4, thus blocking Th2 recruitment (Figure 2B) (18). Conversely, the homeostatic chemokine CCL14/HCC1 circulates in plasma at elevated concentrations as an inactive form, and becomes fully active only after amino terminal processing mediated by proteases associated with inflammatory reactions (19).

4.3. Regulation at the level of receptor expression

The presence of the appropriate receptors in different cell populations dictates the spectrum of action of different chemokines in that chemokine receptors, as their ligands, are subjected to expression control, so that several receptors are detected exclusively in specific cell states. As an example, the dendritic cells maturation process is characterized by a complete switch of the chemokine receptors' expression pattern, with downregulation of receptors for inflammatory chemokines (CCR2 and CCR5 among others) and selective upregulation of CCR7, which drives the mature cell to the draining lymph node. As a general rule, pro- and anti-inflammatory mediators display divergent effects on agonist production and receptor expression, such as in the case of the CCL2/MCP-1-CCR2 axis on monocytes and dendritic cells (Figure 2A) (20).

4.4. Chemokine decoy receptors

Chemokines guide cell migration by controlling multiple biochemical pathways acting in concert. Upon chemokine binding to their specific receptors the Gα and Gβγ subunits of G protein dissociate and activate the production and release of second intracellular messengers. There are also evidences that chemokines can induce G protein-independent signaling pathways, including JAK/STAT activation (21).

The leukocyte activation status plays a crucial role in the definition of chemokine receptors' signaling activity. In certain conditions chemokine receptors are uncoupled by the signaling machinery, and are converted in functional decoys (22). Moreover, beside 'canonical'

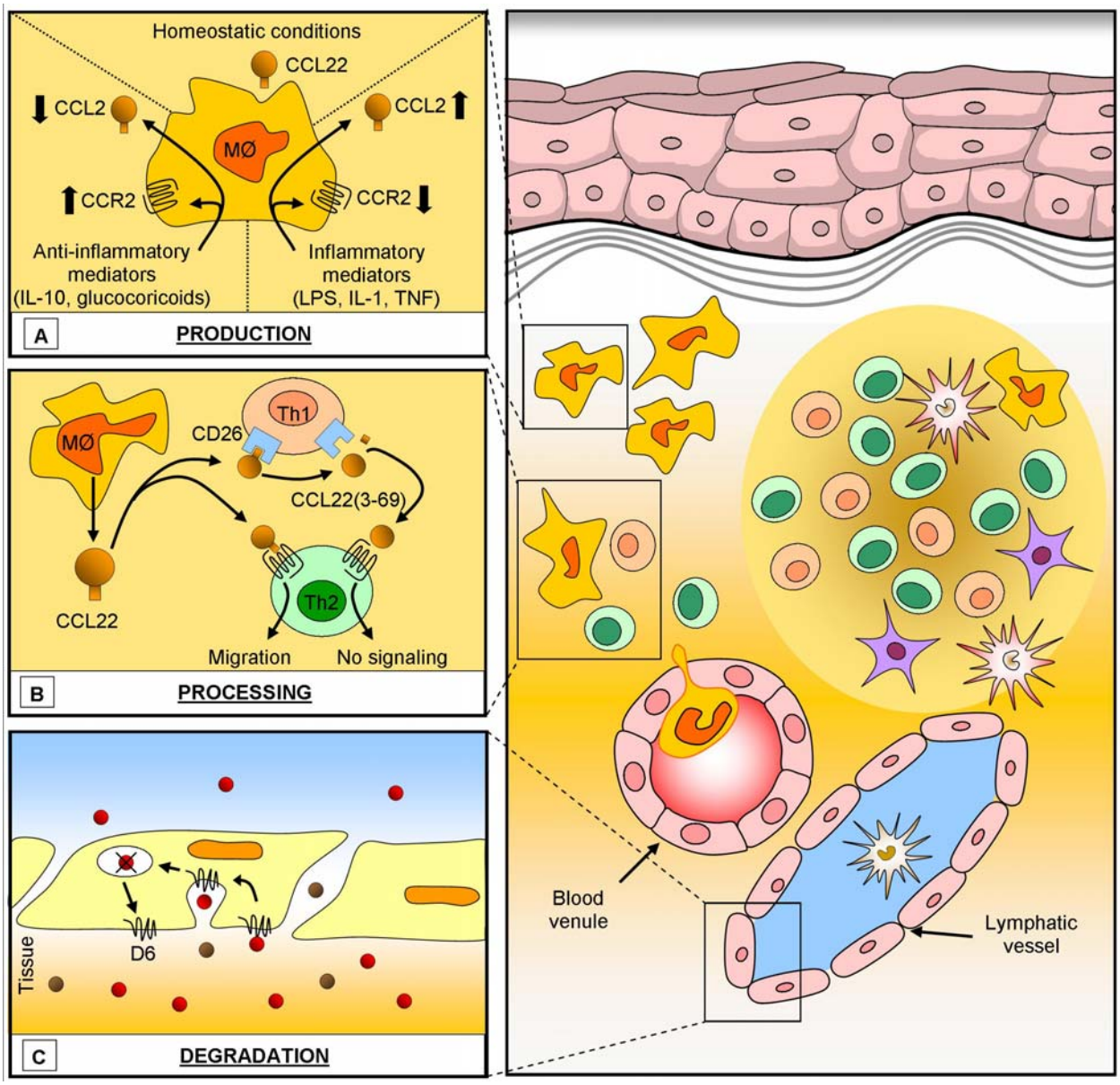

Figure 2. Chemokine regulation. Chemokine regulation occurs at various levels. In terms of production, homeostatic chemokines are constitutively expressed and control leukocyte homing in normal conditions, while inflammatory chemokines and their receptors undergo opposite regulation by pro- and anti-inflammatory mediators (panel A). In certain cases chemokines undergo post-translational processing by proteases, as exemplified by the Th1-mediated CD26-dependent processing of CCL22/MDC that impairs receptor binding and strongly affects chemokine biological activity (panel B). Finally, chemokines can be internalized and degraded by chemokine decoy receptors, such as D6 receptor expressed on draining lymphatic vessels (panel C). During an inflammatory process these events act in a coordinated manner in order to finely tune the local inflammatory process and the lymph node reaction.

chemokine receptors, which are usually coupled to intracellular signals leading to cell migration, a separated group of 'atypical' receptors have been described which bind their cognate ligand with high affinity and specificity but, differently from 'canonical' chemokine receptors, appear to be structurally uncoupled from G proteins not inducing any detectable intracellular signal after chemokine binding. Conversely, in some cases the ligand is transported across the cytoplasm and released on the other side of the cell (*transcytosis*), while in other cases it is internalized and degraded (Figure 2C). Recent *in vivo* evidence clearly indicates that the biological function of this class of receptors is to compete with signaling receptors for the chemokine, sequestering and targeting it to degradation, thus acting as negative regulators of inflammation. For this reason this group previously referred to as "silent"

chemokine receptors have been renamed chemokine *decoy* receptors (23), and it includes at present the molecule DARC (Duffy Antigen Receptor for Chemokines), a promiscuous receptor for CXC and CC chemokines), D6, a receptor selective for CC inflammatory chemokines, and CCX CKR, which selectively targets CC homeostatic chemokines.

D6 is the best characterized chemokine decoy receptor (24). It is a typical chemokine receptor, but like DARC, it lacks sequence motifs that are critical for the G-protein coupling and signaling functions of chemokine receptors, such as the DRY motif in the second intracellular loop as well as the TXP motif in the second transmembrane domain. D6 binds a broad range of ligands that includes most of the inflammatory CC chemokines (agonists of CCR1-CCR5). Nevertheless, D6 has some binding selectivity, in that among inflammatory CC chemokines, it interacts with the non-allelic variant CCL3L1/MIP1αP but not with CCL3/MIP1αS, it distinguishes between the active form and the amino-terminal CD26-processed inactive forms of CCL22/MDC, and does not recognize homeostatic CC chemokines as well as chemokines of other families. D6 is expressed at very low levels by circulating leukocytes, but it is selectively expressed at high levels by endothelial cells of lymphatic afferent vessels in the skin, gut and lungs (25) and in the placenta, where it is present on invading trophoblast cells, on the apical side of syncytiotrophoblast cells and on decidual macrophages (26). D6 does not mediate conventional signaling activities, nor facilitates chemokine transfer through the cell monolayer. Conversely, the presence of D6 consistently results in the degradation of appropriate ligands (27). In fact, this receptor seems to be particularly suited to function as a chemokine scavenger, as it is mainly localized in intracellular stores associated with early and recycling endosomes, it is constitutively internalized in a ligand-independent way through a β-arrestin-dependent clathrin-coated pits mediated pathway (28), and is rapidly recycled on the cell membrane. Differently from conventional chemokine receptors, D6 internalization is a phosphorylation-independent event, and requires a carboxy-terminal region of acidic amino acids that is not found in other chemokine receptors that mediates constitutive interaction with β-arrestin. D6 is particularly efficient at internalizing chemokines, which are then rapidly dissociated and degraded during vesicle acidification, leaving the receptor free to recycle to the cell surface (29). Thus, in *in vitro* settings D6 does not mediate signaling activities or support chemokine transcytosis, but behaves as a decoy receptor that scavenges inflammatory CC chemokines acting as "tapis roulant" that cycles continuously and independently from ligand engagement. D6-/- mice have been generated, and the data obtained in different models are consistent with a role of D6 as a chemokine scavenger *in vivo*. D6 knockout mice have an exacerbated inflammatory response induced following application of phorbol ester to the skin or by subcutaneous injection of complete Freund's adjuvant (CFA) (30, 31). In the former model, inflammation is initiated by TNF and it is then sustained by pro-inflammatory chemokines, with a prominent inflammatory infiltrate that includes T cells, mast cells and neutrophils. Keratinocyte proliferation and neovascularization were also observed, leading to the development of psoriasiform-like lesions. In the second model, D6 knockout animals showed increased inflammatory response at early time points characterized by prominent necrosis and neovascularization that evolved into macroscopic granuloma-like lesions and hyperplasia of draining lymph nodes. Increased levels of inflammatory CC chemokines were detected locally in both models, and pretreatment with blocking antibodies specific for chemokines prevented the development of lesions. Interestingly, the increased local inflammation observed in D6 knockout mice resulted in an impaired specific immune response and protection in an encephalomyelitis model (32). Differently from wild type mice, subcutaneous immunization with myelin oligodendroglial glycoprotein (MOG) peptide 35–55 in CFA led to a marked local inflammatory infiltrate in D6 knockout mice, with $CD11c^+$ cells becoming 'trapped' in aggregates associated with microabscesses, and this altered response protects mice from developing the disease. Recent results also highlight an important role of D6 in placental biology. Exposure of D6 knockout pregnant mice to lipopolysaccharide (LPS) or phospholipids-specific autoantibodies resulted in increased leukocyte infiltration of the placenta and a consequent increase in the rate of fetal loss, which could be prevented by blocking inflammatory chemokines (26). In conclusion, *in vitro* and *in vivo* data strongly support a decoy function for D6, which both in lymphatic vessels and in the placenta acts as a chemokine scavenger and prevents excessive inflammation.

5. THE ROLE OF CHEMOKINES IN HOMEOSTASIS AND IN PATHOLOGY

Leukocyte recruitment, accumulation, and activation are key events in immune-mediated disorders and several animal models, genetic evidences, and clinical studies have now clearly shown the importance of chemokines and chemokine receptors in the pathogenesis of these diseases (for a recent review refer to (33) and references therein).

5.1. Chemokines in homeostatic conditions

Constitutive presence of chemokines in lymph node, thymus, and bone marrow, suggests a regulatory role in normal leukocyte production and distribution, and the phenotypes of mice with targeted disruption of chemokines or chemokine receptor genes are consistent with this hypothesis (34). Thus, CXCR2 knockout have massive expansion of neutrophils and B cells; CXCL12/SDF-1 knockout have severe reduction of myeloid progenitors in the bone marrow, but not in the liver and absent B cell lymphopoiesis; CXCR5 knockout do not develop inguinal lymph nodes or B cell areas in secondary lymphoid tissues and CCL11/eotaxin knockout have increased circulating eosinophils.

5.2. Chemokines and inflammation

Complex patterns of chemokine expression have been correlated with many human inflammatory diseases (1). To distinguish causation from mere association, many

investigators are turning to immunologically and genetically manipulated mice and mouse models of human disease and there are already some interesting early results.

Consistent with the close association of CXCL8/IL-8 with neutrophil-mediated inflammatory disease, e.g. bacterial pneumonia and ischemia-reperfusion injury (35), neutralization of CXCL8/IL-8 in rabbits affords almost complete protection from multiple inflammatory challenges, including lung ischemia-reperfusion injury, pleuritis, and glomerulonephritis (36). Genetic deletion of the mouse CXCL8/IL-8 receptor homologue CXCR2 causes defective neutrophil recruitment (16). Similarly, the CC chemokine CCL3/MIP-1α is present in pathologic specimens from patients with multiple sclerosis, and immunologic neutralization of CCL3/MIP-1α is highly protective against induction of the mouse model of multiple sclerosis, experimental allergic encephalomyelitis (37). Consistent with earlier studies, knockout mice have revealed the importance of CCL2/MCP-1 and CCR2 in monocyte recruitment. Furthermore, CCR2-deficient mice have reduced susceptibility to experimental atherogenesis, in which monocytes and macrophages play an important role (38). Given the large number of chemokines with similar regulation and leukocyte targets, these results indicating non-redundant roles are surprising. They suggest that, *in vivo*, spatial and temporal regulation may be very complicated and functionally significant.

Macrophages and T cell accumulation in autoimmune and degenerative disorders is associated mainly, but not exclusively, with CC chemokines. CC chemokines also appear to be important in allergic inflammation in diseases such as asthma, based on the local presence of CCL11/eotaxin, CCL7/MCP-3, and CCL5/RANTES and the expression of CCL11/eotaxin receptor, CCR3, on eosinophils, basophils, and Th2 cells, which accumulate at sites of allergic inflammation. In agreement with this, in CCL11/eotaxin knockout mice allergen-induced airway eosinophilia is impaired (39).

CCL2/MCP-1 neutralization and CCR1 and CCR2 genetic deletion cause altered Schistosome egg- or PPD-induced granulomatous inflammation that correlates with abnormal Th1 and Th2 cell responses (40). CCR1 knockout mice have reduced pathophysiologic responses during respiratory syncytial virus infection (41). CCR5 knockout mice have enhanced delayed hypersensitivity reactions and increased humoral responses to T cell-dependent antigenic challenge (42). These results indicate complex and not yet understood roles for each of these molecules that extend the chemokine paradigm beyond simple chemotaxis and suggest a role for chemokines in modulating cytokine production in the inflammatory response.

Consistent with the notion that the chemokine system supports host defense, CCR1 and CCR5 knockout mice have increased susceptibility to inoculation with Aspergillus fumigatus (43) and Listeria monocytogenes (44), respectively. However, neither these nor any of the other chemokine/receptor knockout have increased susceptibility to spontaneous infections, indicating that sufficient redundancy exists within the system for baseline host defense. Thus, therapeutic anti-chemokines that will be eventually developed may profit from the specificity that certain chemokines appear to demonstrate in the amplification of pathologic inflammation versus the redundancy that appears to characterize chemokine regulation of immune function.

5.3. Chemokines and infectious diseases

Although chemokines and chemokine receptors probably evolved as antimicrobial factors, many are exploited by infectious agents to facilitate infection (45). Two models of exploitation have been identified. First, intracellular pathogens exploit cellular receptors for cell entry; for instance, the malaria-causing protozoan Plasmodium vivax uses DARC and HIV-1 uses various chemokine receptors. Second, certain herpesviruses encode pirated chemokine receptors, including US28 (used by human cytomegalovirus), ECRF3 (used by Herpesvirus saimiri), and KSHV GPCR (used by Kaposi's sarcoma associated herpesvirus, also known as human herpesvirus 8). US28 and ECRF3 functions have not been identified yet. KSHV GPCR binds multiple chemokines but it is constitutively active, mitogenic for transfected fibroblasts, and angiogenic, suggesting it may be a growth factor in Kaposi's sarcoma (46).

Microbes also have mechanisms that subvert chemokine action (23). Virally encoded chemokine homologues with broad-spectrum chemokine antagonist activity exist in KSHV (vMIP-I and vMIP-II), as well as in the poxvirus Molluscum contagiosum (MC148), which causes skin lesions noteworthy for lack of inflammation. Broad-spectrum chemokine scavengers that lack homology to chemokines and chemokine receptors are encoded by several orthopoxviruses. Although roles in pathogenesis remain to be determined, these viral molecules may be good leads for developing anti-inflammatory drugs with potentially broad clinical applications.

HIV-1 is an enveloped virus that enters the cells by receptor-dependent membrane fusion. The viral fusion determinant is the envelope glycoprotein gp120 and gp41 heterodimer, the product of the Env gene. The primary cellular receptor for all strains of HIV-1 is CD4, but strain-specific chemokine receptors are required as coreceptors for fusion and entry (47). Although virtually all HIV-1 isolates can infect peripheral blood mononuclear cells *in vitro*, two major classes differ in their ability to infect T cell lines versus primary macrophages *in vitro*. The difference is principally due to preferential usage of CXCR4 or CCR5 as coreceptors for T cell line and macrophage infection, respectively. The clinical significance of this observation is supported by the strong but unexplained correlation of CCR5 (R5) strains with both primary infection and the period of clinical latency, and the correlation of more virulent CXCR4 (X4) strains with immune system collapse and AIDS. Many primary clinical isolates are dual-tropic and can use either CCR5 or CXCR4 to infect cells. Many other chemokine receptors and related orphan receptors also have HIV-1 coreceptor activity *in vitro*, including

CX3CR1, CCR8, CCR2, CCR3, STRL33/BONZO, GRP1, GPR15, and apj. Remarkably, CMV US28 is also an HIV-1 coreceptor, suggesting a mechanism for a commensal relationship for these viruses in coinfected individuals. Of these "alternative coreceptors", function in primary cells has been shown only for CCR3, specifically in microglial cells demonstrated using a blocking antibody. CCR5 and CXCR4 have also passed this test in primary cells with a variety of blocking agents, including agonists, antagonists, and antibodies (48). Only for CCR5, however, a clear-cut role in pathogenesis has been demonstrated, through discovery of a mutant allele bearing a 32-base-pair deletion (CCR5Δ32) in the open reading frame, which encodes a truncated, inactive receptor. The Δ32/Δ32 genotype is highly protective against initial infection, it is found 20-fold less frequently among HIV-1-infected people than in the general population and 6-fold more frequently among highly exposed HIV-1-negative individuals than among HIV-1-infected individuals. However, few exposed HIV-1-negative individuals are Δ32/Δ32, indicating that other resistance factors must exist. The protective effect is independent of the route of transmission. The wt/Δ32 genotype does not protect against initial infection, but in some studies it has been associated with a mean two-year delay in progression to AIDS and has shown increased frequency among long-term nonprogressors. This correlates with reduced CCR5 on T cells from wt/Δ32 individuals (around 10% of wt/wt controls), which may be partly due to dysfunctional heterodymers composed of normal and truncated receptor subunits. CCR5 Δ32 appear to have originated in northeastern Europe, and it occurs with an average allelic frequency of 10% in North American Caucasians; it is not found in native Asians and Africans. Many others polymorphism are found in chemokine and chemokine receptor genes, and some are known to affect HIV-1 pathogenesis (49). One is a rare protein-truncating mutation of CCR5 (m303) and is presumed to act like Δ32. The others affect the rate of disease progression but may not affect susceptibility to initial infection, and the mechanisms are unclear. One of these polymorphisms causes a conservative aminoacid change in CCR2 (V64I), and the other two are single nucleotide polymorphisms in the 3' untranslated region of CXCL12/SDF-1 mRNA and the CCR5 promoter (59029A/G) that act as recessive traits. The promoter polymorphism has a particularly strong effect and is very common in all racial group tested. The fact that CCR5 deficiency causes HIV-1 resistance despite the presence of many other coreceptors could be due to multiple factors, including CCR5's relative efficiency as a coreceptor, sequestration of other coreceptors from leukocyte targets in rectal and vaginal mucosa, preferential suppression of X4 strains from CXCR4. Similarly, CCR5 agonists could regulate the rate of progression to AIDS by blocking R5 viruses from CCR5 interaction during the period of clinical latency. Experimental support for this possibility was provided by the demonstration that the CD8 T cell-derived soluble factors able to suppress R5 (but not X4) HIV-1 replication were represented by the CCR5 ligands CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES. Moreover, certain exposed HIV-1-negative patients have relatively high levels of CCR5 ligand production. These observations could conceivably be translated into clinical benefit by developing receptor antagonists as therapeutic blocking agents. This approach has to take in account the recently described protective role of CCR5 in the infection by West Nile virus, a pathogen causing fatal encephalitis. Blocking CCR5 in HIV infected patients could increase the risk of West Nile virus disease (50). Moreover, CCR5 ligands are able to block R5 infection of primary lymphoid tissue *ex vivo* only at high concentrations difficult to achieve *in vivo*. Also, chemokines enhance HIV-1 replication in certain contexts, such as lymph nodes treated with low concentrations of CCR5 agonists.

The HIV-1 coreceptor story strongly parallels malaria pathogenesis, where DARC (a multispecific, non-signaling chemokine binding protein on erythrocytes) is used as a receptor by Plasmodium vivax for cell entry (51). Most Africans lack DARC on erythrocytes (although they express it on other cell types) and are therefore protected from vivax malaria. DARC absence correlates with a mutation in a GATA-1 site in DARC promoter. Although inherited DARC deficiency was probably fixed in African populations by the selective pressure of vivax malaria, HIV-1 could not have been responsible for fixation of HIV-1 coreceptor mutations. Instead, ancestral epidemics caused by microbes that also exploited chemokine receptors for pathogenesis may have been responsible.

5.4. Chemokines and angiogenesis

The ELR motif (see above) divides CXC chemokines into angiogenic (ELR^+) and angiostatic (ELR^-) factors, as assessed by their effects on endothelial cell proliferation and chemotaxis *in vitro* and on the angiogenesis in animal models, such as rat cornea and tumor regression *in vivo* (52, 53). The ELR^- chemokines inhibit not only angiogenic chemokines but also other major angiogenic mediators, such as vascular endothelial growth factor and fibroblast growth factor. Chemokine angiogenic activity is probably mediated by CXCR2, the endothelial receptor for ELR^+ chemokines. On the other hand, it is not clear which receptor (s) are responsible for the angiostatic effect of ELR^- chemokines. A role for heparan sulfate proteoglycan components of the endothelial cell membrane has bee reported, suggesting that the mechanism could reside in displacement of other angiogenic growth factors.

5.5. Chemokines and tumors

Chemokines and chemokine receptors have been shown to affect several aspects of cancer development, including leukocyte infiltration, tumour growth, angiogenesis, and metastasis (for references refer to (54) and citations therein). Different tumours present a different leukocyte infiltrate, recruited by the corresponding chemokines. For example, a selective infiltration of $CCR5^+$ and $CXCR3^+$ T lymphocytes is present in human colorectal carcinoma, while $CXCR3^+$ B cells are found at elevated frequency in the peripheral blood of patients with MALT lymphoma. Several clinical and epidemiological evidences suggest that chemokines can favour tumour development. As an example, in ovarian and breast cancers chemokine levels (CCL2/MCP-1 and CCL5/RANTES) correlate with

macrophage infiltration, lymph node metastasis and clinical aggressiveness (55). Chemokine receptors also play a fundamental role in tumour progression and metastasis. Melanoma cells express high levels of CXCR2 and also constitutively produce its ligands CXCL1/GROα and CXCL8/IL-8 that stimulate in an autocrine way proliferation and survival. Similarly, prostate cancer cells and glioblastoma cells express both CXCR4 and CXCL12/SDF-1, thus stimulating their proliferation (56). Tumor progression and metastasis involve CXCR4 in particular. CXCR4 expression reflects tumor progression and regulates motility of bladder cancer cells, and it is also associated with lymph node metastasis in oral squamous cell carcinoma, human nasopharyngeal carcinoma, pancreatic cancer, non-small lung cancer, human colorectal cancer (in association with CCR7), and human breast cancer. Taken together, these data clearly demonstrates a relevant function of the chemokine system in several aspects of neoplastic diseases, with an emerging role of CXCL12/SDF-1-CXCR4 axis in the metastatic process (57).

6. PERSPECTIVE

Enough data have been collected to conclude confidently that members of the chemokine system are of major importance in specific aspects of development, inflammation and susceptibility to infectious diseases. Whether these findings can be translated into clinical benefit will depend on the balance of redundancy versus specificity in the system. The notion of master chemokines and chemokine receptors may be realistic, at least in certain settings such as CXCL8/IL-8 in acute inflammation and CCR5 in HIV-1 infection, and these molecules are attractive targets for drug development.

7. ACKNOWLEDGEMENTS

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Abbreviations: TNF, Tumour Necrosis Factor; IFN, Interferon; DARC, Duffy Antigen Receptor for Chemokines; HHV8, human herpes virus 8; v-MIP, viral macrophage inflammatory protein; RA, rheumatoid arthritis; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; CX3CL, CX3C chemokine ligand; XCL, C chemokine ligand; CCR, CC chemokine receptor; CXCR, CXC chemokine receptor; CX3CR, CX3C chemokine receptor; XCR, C chemokine receptor

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