Review

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Bias in chemokine receptor signalling

Annelien J.M. Zweemer, Jimita Toraskar, Laura H. Heitman, and Adriaan P. IJzerman

Division of Medicinal Chemistry, Leiden Academic Centre for Drug Research, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands

Chemokine receptors are widely expressed on a variety of immune cells and play a crucial role in normal physiology as well as in inflammatory and infectious diseases. The existence of 23 chemokine receptors and 48 chemokine ligands guarantees a tight control and fine-tuning of the immune system. Here, we discuss the multiple regulatory mechanisms of chemokine signalling at a systemic, cellular, and molecular level. In particular, we focus on the impact of biased signalling at the receptor level; an emerging concept in molecular pharmacology. An improved understanding of these mechanisms may provide a framework for more effective drug discovery and development at a target class that is so relevant for immune function.

Regulation of the chemokine system

Chemokines are the most important regulators of leukocyte trafficking and play a central role in the immune system [1]. They act via abundantly expressed chemokine receptors, which belong to the family of G protein-coupled receptors (GPCRs) (Box 1), on a wide variety of immune cells. Activation of these chemokine receptors induces migration and differentiation of immune cells, which both are essential processes during innate and adaptive immune responses [2].

The chemokine-directed immune response involves a complex network of reactions that are carefully fine-tuned at multiple levels throughout the body (Figure 1). At the systems level this involves spatiotemporal and tissue-specific expression of chemokine receptors and their ligands. At the cellular level the chemokine receptor signal can be modulated by coexpression of many differentially expressed proteins on immune cells. Finally, there is growing evidence of biased signalling at the molecular level for chemokine receptors, which implies that different chemokine ligands activate different intracellular pathways although binding to the same receptor.

With regard to this bias at the receptor level, novel mechanistic insights have been attained lately due to the advances in X-ray crystallography and NMR methods to resolve the structure of membrane proteins, such as GPCRs. Several structures of chemokine receptors have been elucidated now, among which are chemokine CXC receptor (CXCR)1, CXCR4 and chemokine CC receptor (CCR)5 [3–5]. In addition, for the serotonin 5-hydroxytryptamine $(HT)_{1B/2B}$ and the β_2-adrenergic receptors a structural basis for biased signalling was reported [6,7]. Similar mechanisms for ligand bias are likely to be present for the family of chemokine receptors, because these are particularly prone to biased signalling due to the presence of multiple endogenous chemokine ligands.

So far there has only been limited success in clinical trials targeting chemokine receptors. We propose therefore to consider chemokine regulation and bias at multiple levels in order to better understand their intricacies. Thus, in this review we present a summary of chemokine receptor signalling at a systems, cellular, and molecular level. Immunologists should be aware of the bias that can be introduced at a molecular level, whereas pharmacologists need to keep in mind that their target molecule could be modulated or expressed differently at a systems level.

Regulation of chemokine expression and receptor activation

The human chemokine system consists of ~23 receptors and 48 ligands [IUPHAR/BPS Guide to Pharmacology, http://www.guidetopharmacology.org, accessed on 07-02-2014], of which the classically signalling chemokine receptors are presented in Figure 2. Most chemokine receptors can be activated by multiple chemokines, and one chemokine often has the ability to activate multiple receptors. Although previously regarded as redundant, the unique expression patterns of the various chemokines suggest that they form the basis for a specific and fine-tuned functioning of the immune system [1]. This is not only important in normal physiology, but also during certain immunopathological disease states, as illustrated by the CCR2 receptor and its ligands. CCR2 can be activated by the chemokine ligands chemokine CC ligand (CCL)2/monocyte chemotactic protein (MCP)-1, CCL7/MCP-3, CCL8/MCP-2, CCL11/eotaxin, CCL13/MCP-4, and CCL16/human CC chemokine (HCC)-4. Most studies have been focused on the CCL2–CCR2 interaction because CCL2 is the endogenous ligand with the highest affinity for CCR2. Nevertheless, in infectious diseases, CCL7 has been found to be crucial for monocyte recruitment to inflammatory sites mediated through CCR2 [8]. An example of distinct expression patterns observed in immunopathology is the regulation of the CCR4 ligands CCL17/thymus- and activation-regulated chemokine (TARC) and CCL22/macrophage-derived chemokine (MDC), which are not expressed in healthy skin tissue [9]. However, in inflamed skin lesions, CCL17 is detected on endothelial cells, whereas CCL22 is only presented by dendritic cells [9]. This distinct chemokine

Corresponding author: IJzerman, A.P. (ijzerman@lacdr.leidenuniv.nl).
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Box 1. Chemokine receptors as GPCRs

GPCRs

- With >800 members, GPCRs are the largest family and most diverse group of cell surface receptors and the most common target for therapeutic drugs [79].
- The GPCR structure consists of an extracellular N terminus, an intracellular C terminus, and seven transmembrane (TM) helices, connected by three cytoplasmic and three extracellular loops [80].
- Ligand binding mostly takes place in a pocket formed by the seven helices close to the extracellular side of the receptor; it induces a conformational change at the intracellular side of the receptor that results in receptor activation and subsequent signalling [81].
- At the intracellular side different effector proteins can bind and transduce signals, among which are G proteins and β-arrestins [82].

Chemokine receptors

- Chemokine receptors belong to the class A rhodopsin-like family of GPCRs.
- 23 different chemokine receptors have been identified that can be activated by ~48 chemokine ligands [IUPHAR/BPS Guide to Pharmacology, http://www.guidetopharmacology.org, accessed on 07-02-2014].
- Four subclasses of chemokine ligands have been identified on the basis of the pattern of conserved cysteine residues (C, CC, CXC, and CX_3C) [83].
- Chemokine receptors have been classified as C, CC, CXC, and CX_3C receptors based on the chemokine subclass ligand that they bind.
- Most chemokine receptors bind multiple chemokines, and most chemokines can bind to and activate multiple chemokine receptors.
- The chemokine receptors ACKR1 (DARC), ACKR2 (D6), ACKR3 (CXCR7), and ACKR4 (CCX-CKR) are so-called decoy receptors that predominantly scavenge chemokine ligands from the extracellular environment, although some of these also couple to β-arrestins [16].

expression pattern has been demonstrated in diseases ranging from psoriasis to atopic dermatitis, therefore, this could be a general feature underlying the disease state. In general the balance, timing, and pattern of chemokine expression appears to regulate the generation of immune-cell-specific responses in health and disease [10].

In addition to the difference in release and production of chemokines among various tissues, their *in vivo* availability also depends on the interaction of chemokines with specific glycosaminoglycan (GAG) chains that are presented at the cell surface as part of membrane proteoglycans. The binding of chemokines to GAGs allows immobilization, accumulation, and retention of chemokines on cell surfaces near their sites of production in order to provide directional signals to migrating cells [11]. In addition, GAG interactions are involved in the transport of chemokines across cell surfaces. GAGs may selectively bind chemokines and therefore fine-tune the immune response, because they display varying affinities for specific chemokines and are differentially expressed in time and location on specific cell types and tissues [12]. Furthermore, cells and tissues can alter the expression of GAGs in pathophysiology. This has been observed upon inflammatory stimuli in diseases of the gastrointestinal tract as well as in multiple different tumours [13,14]. GAGs might even be directly involved in signalling, because their attached core proteins that span the membrane can undergo tyrosine phosphorylation and thereby contribute to signal transduction, as reported for CXCL12/SDF-1 and the proteoglycan syndecan-4 [15]. Although they are a crucial factor for chemokine signalling, the exact functional consequences of chemokine–GAG interactions and the level of specificity are still largely speculative.

Not only GAGs can alter the availability of chemokines, but also chemokine receptors themselves. A certain group of chemokine receptors, known as atypical chemokine receptors (ACKRs) [16], have been proposed to act mainly as chemokine ligand scavengers [17,18]. Furthermore, under certain circumstances the G protein-coupled chemokine receptors have been demonstrated to become uncoupled from G protein signalling. For example, dendritic cells and monocytes treated with anti-inflammatory interleukin (IL)-10 express 'uncoupled' or 'nonsignalling' CCR1, CCR2, and CCR5, which can scavenge their corresponding inflammatory chemokines *in vitro* as well as in mice [19]. Another study demonstrated both *in vitro* and *in vivo* that apoptotic leukocytes express 'silent' CCR5 receptors, scavenging CCR5 ligands, and thereby contributing to the resolution of inflammation in a mouse model of peritonitis [20]. Therefore, expression of a certain chemokine receptor does not always imply a contribution to the disease state. In fact, one might speculate that a pharmacological blockade of these receptors can increase free chemokine levels and therefore result in enhanced pathology.

Altogether, the examples above illustrate that the expression of chemokines and their receptors varies over time and between different conditions, and studies of mechanisms and outcomes associated with this differential expression in several disease states have been reviewed previously [21,22]. As noted above, it is clear that expression of chemokines and their receptors does not necessarily imply a role as stimulator or enhancer of a pathophysiological state, which is an important factor to consider while developing antagonists targeting the chemokine system.

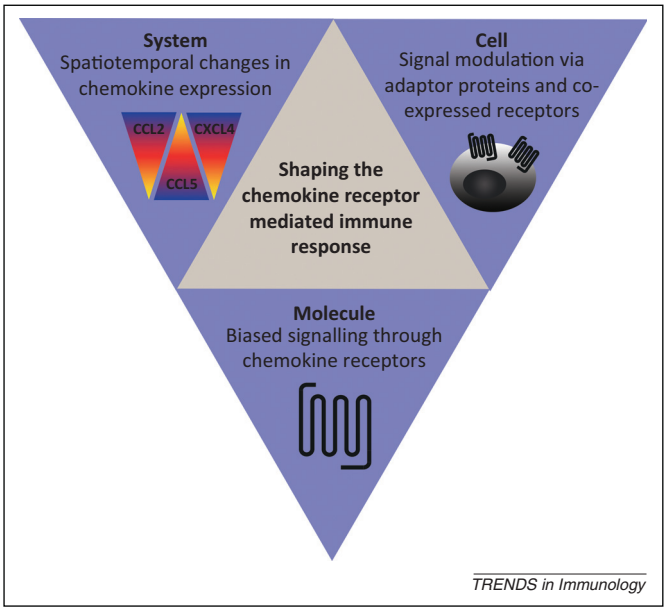

Figure 1. Schematic representation of the structure of this review. The chemokine receptor-mediated immune response is discussed at a systems, cellular, and molecular level.