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Review

Biased signaling of G protein-coupled receptors – From a chemokine receptor CCR7 perspective

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ABSTRACT

Chemokines (chemotactic cytokines) and their associated G protein-coupled receptors (GPCRs) work in a concerted manner to govern immune cell positioning in time and space. Promiscuity of both ligands and receptors, but also biased signaling within the chemokine system, adds to the complexity of how the cell-based immune system is controlled. Bias comes in three forms; ligand-, receptor- and tissue-bias. Biased signaling is increasingly being recognized as playing an important role in contributing to the fine-tuned coordination of immune cell chemotaxis. In the current review we discuss the recent findings related to ligand- and tissue-biased signaling of CCR7 and summarize what is known about bias at other chemokine receptors. CCR7 is expressed by a subset of T-cells and by mature dendritic cells (DCs). Together with its two endogenous ligands CCL19 and CCL21, of which the carboxy terminal tail of CCL21 displays an extraordinarily strong glycosaminoglycan (GAG) binding, CCR7 plays a central role in coordinating the meeting between mature antigen presenting DCs and naïve T-cells which normally takes place in the lymph nodes (LNs). This process is a prerequisite for the initiation of an antigen-specific T-cell mediated immune response. Thus CCR7 and its ligands are key players in initiating cell-based immune responses. CCL19 and CCL21 display differential interaction- and docking-modes for CCR7 leading to stabilization of different CCR7 conformations and hereby preferential activation of distinct intracellular signaling pathways (i.e. ligand bias). In general CCL19 seems to generate a strong temporal signal, whereas CCL21 generates a weaker, but more persistent signal. Tissue differential expression of these two ligands, and the generation of a third ligand "tailless-CCL21", through DC specific protease activity (tissue bias), orchestrates DC and T-cell LN homing and priming, with each ligand serving overlapping, but also distinct roles.

1. Introduction-bias in the chemokine system

The chemokine system consists of 50 chemokines and 25 chemokine receptors and is characterized by a high degree of promiscuity, meaning that certain chemokines interact with more than one receptor an vice versa as summarized in the overview presented in Fig. 1 (Bachelerie et al., 2014). Promiscuity gives rise to ligand-, receptor-, and tissue-bias as reported in CCR7 (Table 1) as well as various other chemokine receptor systems (Table 2) and refers to 1) different ligands inducing bias at the same receptor, 2) the same ligand inducing biased responses in different receptor contexts and finally 3) differential expression of receptors and ligand by different tissues (Fig. 2) (Steen et al., 2014a).

Understanding the molecular mechanisms controlling biased signaling at GPCRs is a prerequisite for being able to selectively target receptor functions as opposed to unselectively antagonizing receptor signaling as a whole. Such information is needed in the development of drugs targeting central immune cell receptors like CCR7 that control both acquired and innate immune functions; thus side-effects is anticipated to be less severe with drugs that antagonize only a subset of ligands or specific signaling pathways.

2. CCR7; a receptor controlled by three ligands expressed in a tissue biased manner

The two CCR7 ligands CCL19 and CCL21 are expressed in an overlapping but not identical set of tissues. Whereas both chemokines are expressed by stromal cells of the LN (Luther et al., 2000) and by high endothelial venule (HEV) cells (with CCL21 being the predominant here) (Luther et al., 2002), afferent lymphatic vessels exclusively express CCL21 (Luther et al., 2000) and activated DCs produce and secrete CCL19, but not CCL21 (Sallusto et al., 1999). This difference in expression pattern gives rise to tissue bias. CCL21 is believed to be the main driver of LN homing for both T-cells (via HEVs) and DCs (via afferent lymphatic

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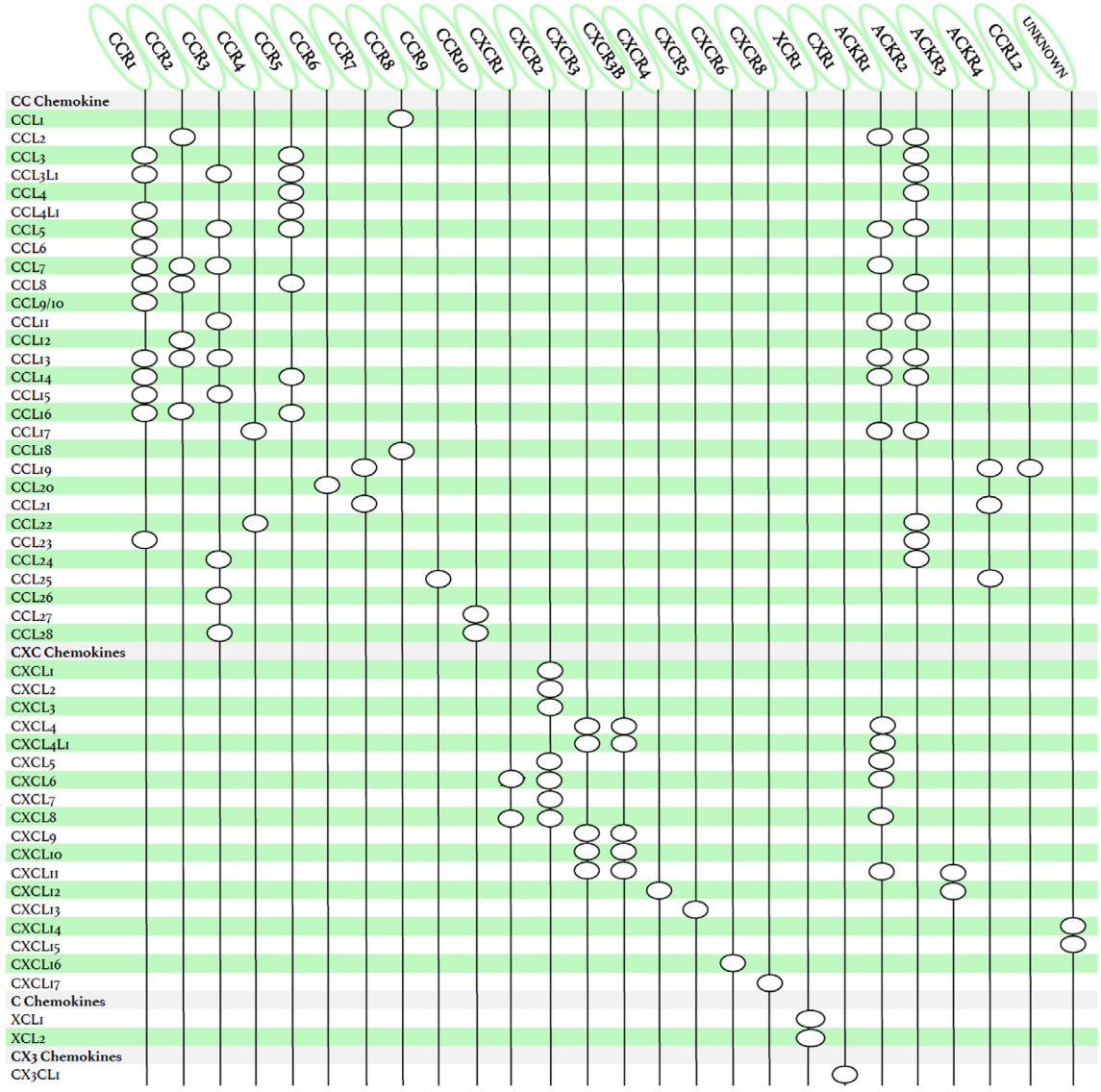

Fig. 1. Overview of chemokine receptor/ligand pairs. The promiscuity of this receptor system is indicated. The receptors for the chemokines CXCL14, and -15 are still unidentified. Adapted and updated from Steen et al. (Steen et al., 2014a). CXCL4L1 is a non-allelic gene variant of CXCL4, binding the same receptors but displaying distinct properties (Van et al., 2015). The receptor for CXCL17 has recently been identified as CXCR8, also known as GPR35 (Maravillas-Montero et al., 2015).

vessels), but also to coordinate intranodal positioning of both cell types through the action of ACKR4 (aka CCR1L1 or CCR11), a scavenger receptor for CCL21 that shapes intranodal CCL21 gradients (Ulvmar et al., 2014). CCL19, produced by activated DCs, is believed to be important for the short-lived interaction between DCs and T-cells during the DC:T-cell scanning (Kaiser et al., 2005; Muthuswamy et al., 2010). Restriction of ligands to specific tissues (tissue bias) is an important factor, as illustrated with premature CCL19 secretion from DCs that have not yet migrated to LNs, a situation that seems to impair homing. This scenario can be a problem in cell-based therapies where patient DCs are primed with antigen in vitro and then re-injected in the patient for intended generation of an antigen-specific T-cell response upon LN homing (Hansen et al., 2016). Also, ACKR4 expressed by stromal cells of the skin functions to remove any skin-derived CCL19, and disturbance of this system negatively affects LN homing of DCs (Bryce et al., 2016).

Despite both being endogenous ligands for CCR7, CCL19 and CCL21 only share 32% sequence identity. Further, CCL21 has a 37 amino acid long C-terminal tail, rich in positively charged residues, that confers high affinity for negatively charged molecules of the extracellular matrix (ECM), including GAGs (de Paz et al., 2007; Patel et al., 2001). Importantly, CCL19 does not have this C-terminal basic extension. CCL21 exist in two forms, each with different ligand properties: full length CCL21 and a naturally occurring C-terminally truncated version, tailless-CCL21, generated through a DC-specific protease activity (Schumann et al., 2010). The fact that the tailless-CCL21 resembles neither CCL19 nor CCL21 with regard to signaling properties (Hauser et al., 2016a; Hjorto et al., 2016), indicates that ligand bias between CCL19 and CCL21 is not only determined by the GAG-binding C-terminus of CCL21, and rather defines tailless-CCL21 as a functionally distinct ligand with specific properties. That new versions of chemokines can be produced via enzymatic action is not new, in fact the action of dipeptidyl peptidase IV (DPP4) as well as various matrix metalloproteases (MMPs) are known to influence the action of many chemokines, making them stronger or weaker agonists or even changing their action from agonistic to antagonistic (Metzemaekers et al., 2016), adding to the general complexity of *tissue bias*. Although some functions of the C-terminally truncated

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