

## INVITED REVIEW

## Concepts of GPCR-controlled navigation in the immune system

Tim Lämmermann<sup>1</sup> | Wolfgang Kastenmüller<sup>2</sup> <sup>1</sup>Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany<sup>2</sup>Institute of Systems Immunology, University of Würzburg, Würzburg, Germany**Correspondence**

Tim Lämmermann, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany.

Email: laemmermann@ie-freiburg.mpg.de and Wolfgang Kastenmüller, Institute of Systems Immunology, University of Würzburg, Würzburg, Germany.  
Email: wolfgang.kastenmueller@uni-wuerzburg.de**Funding information**

H2020 European Research Council, Grant/Award Number: CoG 819329 and StG 715890

**Summary**

G-protein-coupled receptor (GPCR) signaling is essential for the spatiotemporal control of leukocyte dynamics during immune responses. For efficient navigation through mammalian tissues, most leukocyte types express more than one GPCR on their surface and sense a wide range of chemokines and chemoattractants, leading to basic forms of leukocyte movement (chemokinesis, haptokinesis, chemotaxis, haptotaxis, and chemorepulsion). How leukocytes integrate multiple GPCR signals and make directional decisions in lymphoid and inflamed tissues is still subject of intense research. Many of our concepts on GPCR-controlled leukocyte navigation in the presence of multiple GPCR signals derive from *in vitro* chemotaxis studies and lower vertebrates. In this review, we refer to these concepts and critically contemplate their relevance for the directional movement of several leukocyte subsets (neutrophils, T cells, and dendritic cells) in the complexity of mouse tissues. We discuss how leukocyte navigation can be regulated at the level of only a single GPCR (surface expression, competitive antagonism, oligomerization, homologous desensitization, and receptor internalization) or multiple GPCRs (synergy, hierarchical and non-hierarchical competition, sequential signaling, heterologous desensitization, and agonist scavenging). In particular, we will highlight recent advances in understanding GPCR-controlled leukocyte navigation by intravital microscopy of immune cells in mice.

**KEYWORDS**chemokines, dendritic cells, *in vivo* imaging, neutrophils, T cells**1 | INTRODUCTION**

Innate and adaptive immune responses depend on the coordination and spatiotemporal control of leukocyte dynamics between and within lymphoid organs and peripheral tissues. A wide spectrum of chemically diverse molecules including small proteins, peptides, lipids, nucleotides, and extracellular matrix fragments can act as chemoattractants, regulating leukocyte motility, migration, chemotaxis, positioning, and cell-cell interaction. Leukocytes perceive these

signals mainly through G-protein-coupled receptors (GPCRs) that, upon agonist binding, induce intracellular promigratory responses.<sup>1,2</sup> Based on the cell type-specific expression of cell surface GPCR type combinations and intracellular GPCR-controlling protein networks, each leukocyte subset has evolved specific trafficking paths to fulfill their designated effector functions in the body.<sup>3-5</sup> Over the last decades, the analysis of GPCR-deficient mouse models has advanced our understanding of how specific GPCR signals determine immune cell positioning and compartmentalization *in situ*. Recent work now also started to shed light on the establishment of chemokine patterns and chemotactic fields that guide leukocytes in mammalian

This article is part of a series of reviews covering Global positioning by Chemokines and other Mediators appearing in Volume 289 of *Immunological Reviews*.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2019 The Authors. *Immunological Reviews* Published by John Wiley & Sons Ltd

tissues.<sup>6,7</sup> In particular, intravital imaging of control and GPCR-deficient leukocytes in tissues of live anesthetized mice has brought unprecedented insight into the dynamic migration patterns of immune cell subsets in situ.<sup>8,9</sup> Despite these achievements, it is still largely unclear how leukocytes make directional decisions in lymphoid and inflamed organs where they constantly encounter GPCR signals emanating from multiple sources. The detailed mechanisms of how leukocytes sense, integrate, and prioritize between multiple chemotactic fields in situ and how this influences their physiological trafficking are still largely unknown. Dissecting these molecular events experimentally in mammalian tissues is very challenging and demanding. In particular, we are missing important pieces of information (eg, the exact distribution and concentrations of known chemoattractant, GPCR dynamics in leukocytes) and cannot control for unknown chemotactic fields. Due to these current limitations many of the emerging concepts for leukocyte navigation in complex chemoattractant milieus come from studies in controlled in vitro systems<sup>2</sup> or lower model organisms.<sup>10,11</sup> However, these concepts from reductionist models may not always be easily translatable to the complex situation in mammalian lymphoid organs and inflamed peripheral tissues. In recent years, several systematic studies in mice have started to disentangle how multiple GPCR signals shape leukocyte migration patterns in the complexity of mammalian tissues.

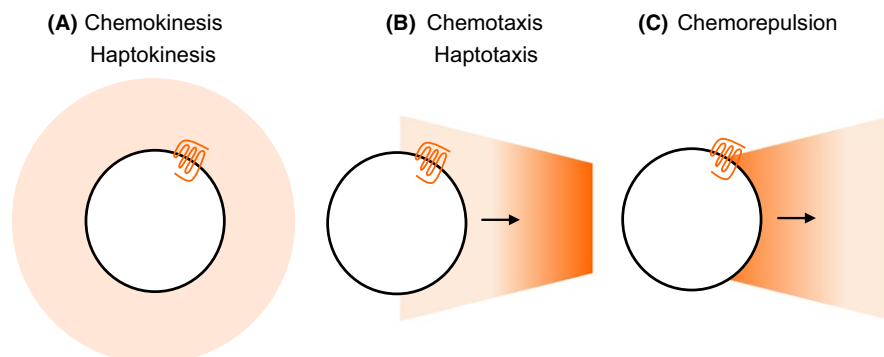
In this review, we will summarize common concepts and mechanisms of GPCR-controlled leukocyte navigation coming from studies with mouse and human leukocytes. We outline the protein networks that control GPCR signaling and GPCR activity and discuss how these may control leukocyte navigation strategies. In particular, we highlight our current knowledge on leukocyte navigation in complex chemoattractant fields and summarize them for three leukocyte subsets (neutrophils, T cells, and dendritic cells [DC]) with different physiological trafficking paths. For each cell type, we will critically evaluate the relevance of current concepts for leukocyte navigation in mouse tissue. While it is very likely that some navigation strategies are only relevant for certain immune cell types and adapted to specific physiological tissue environments, leukocyte subsets may

also share basic strategies for directional decision-making in lymphoid and inflamed tissues of mammals.

## 1.1 | Concepts and mechanisms of GPCR-controlled leukocyte navigation

Many chemokines and chemoattractants are classical inducers of leukocyte polarity, adhesion, and locomotion. They act through GPCRs on intracellular signaling pathways to establish biochemical asymmetry within the cell, preceding the formation of a polarized cell with an actin-rich front and a myosin II-rich rear.<sup>12,13</sup> Key molecular pathways involved in establishing functional polarity downstream of GPCRs in leukocytes depend on phosphoinositide 3-kinases (PI3K) and members of the Rho family of small GTPases.<sup>14</sup> Rac isoforms, Cdc42 and RhoA represent major signal hubs that act through several effector molecules to regulate actin polymerization and actomyosin cytoskeleton. The balanced interplay of these components shapes the typical amoeboid migration mode of each leukocyte subset.<sup>15,16</sup>

In homogeneous fields of soluble chemokines and chemoattractants, most leukocytes undergo self-polarization, before increasing their motility, migration speed, and movement in a random and undirected fashion (*chemokinesis*) (Figure 1A).<sup>17</sup> Chemokinesis is considered a migratory pattern that helps leukocytes scan large tissue volumes. We have learnt from intravital microscopy studies in mice that lymphocytes show such continuous undirected (random-walk) migration in lymphoid organs to enable encounters and interactions with other leukocytes.<sup>9</sup> Together with other determining factors (eg, confinement between stromal tissue elements), uniform chemokine signals critically control chemokinesis by increasing the speed of randomly moving lymphocytes in situ.<sup>18</sup> Once leukocytes perceive external gradients of soluble chemokines and chemoattractants, they can perform directional movement along the concentration gradients of these diffusing attractants (*chemotaxis*) (Figure 1B).<sup>17</sup> Some leukocyte subsets have also been reported to be repelled from high chemoattractant concentrations (*chemorepulsion*), leading to their movement against the gradient (*fugetaxis*).<sup>19</sup> However, the underlying molecular mechanisms of this



**FIGURE 1** General GPCR-controlled migration responses. (A) Homogeneous fields of soluble chemoattractants (chemokinesis) or surface-immobilized chemokines (haptokinesis) can induce leukocyte self-polarization and migration in a non-directed fashion. (B) Gradients of soluble chemoattractants (chemotaxis) or surface-immobilized chemokines (haptotaxis) can direct leukocytes toward higher attractant concentrations along the gradient. (C) Leukocytes have also been observed to perform directed migration against a chemoattractant gradient (chemorepulsion/fugetaxis)

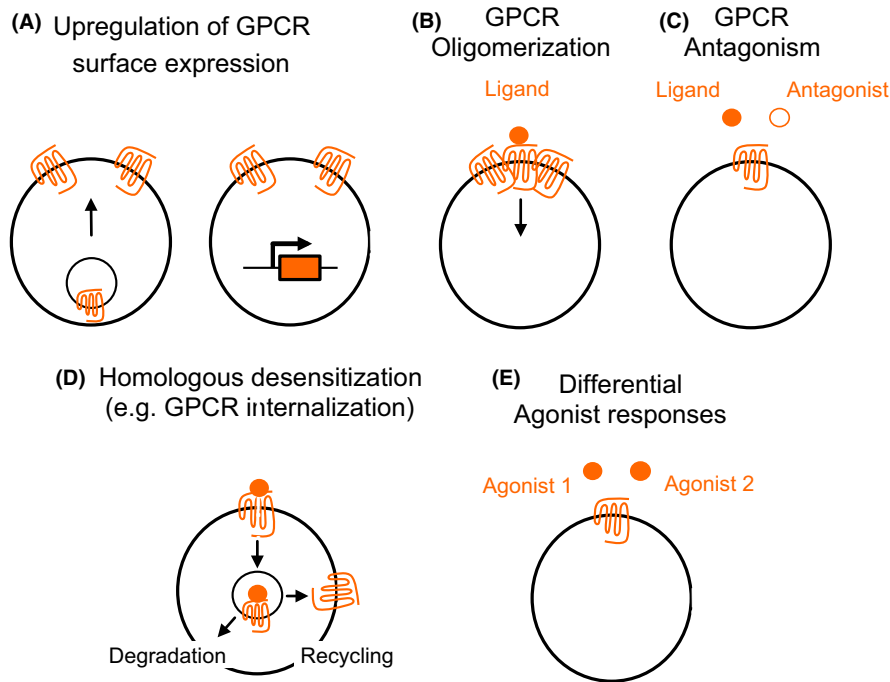
process are still under investigation (Figure 1C).<sup>20,21</sup> Apart from soluble molecular cues, leukocytes also perceive surface-bound attractants as gradients or as homogeneous fields and undergo movement along these surfaces in a directed (*haptotaxis*) (Figure 1B) or undirected manner (*haptokinesis*) (Figure 1A), respectively.<sup>16,22</sup> The aspect of leukocyte navigation along surface-bound attractants is particularly relevant for chemokines with binding capacities to extracellular glycosaminoglycans (GAGs), which decorate many stromal cell elements in tissues.<sup>22,23</sup> This form of imprinting non-diffusible chemokine gradients into the tissue has been shown critical for leukocyte trafficking across the blood endothelium and entry into lymphatic vessels.<sup>6,23</sup> The terms “chemotaxis” and “haptotaxis” subsume a spectrum of navigation strategies, which leukocytes can use to follow attractant gradients.<sup>24</sup> When perceiving an external attractant gradient, some leukocytes (eg, unstimulated neutrophils in culture dish) undergo a full morphological change from unpolarized shape to the establishment of a front-back axis, which then leads to directional movement along the gradient. In contrast, other leukocytes already migrate with pre-established polarity (eg, DCs and neutrophils in 3D), before they steer along the gradient by redirecting their migration path. Alternatively, prepolarized leukocytes may move along a gradient without redirecting their path and start to accelerate the closer they get to the attractant source (eg, neutrophils in response to dying cells). Once at the highest attractant source, leukocytes may also actively decelerate and slow down their migration.<sup>24</sup> As evidenced from intravital microscopy of select leukocyte subsets, we know that this spectrum of chemotactic responses also occurs in mouse tissues. While we acknowledge that chemokines and chemoattractants are central for many aspects of these migratory responses, we are missing a detailed understanding about how these signals shape the navigation of leukocytes *in situ*. Is the cellular movement guided by one or more attractant signal(s)? How do leukocytes navigate in complex gradient fields and sense the right direction? How do leukocytes sense their final destination and know where to stop? Are there inherent differences between leukocyte subsets in their navigation strategies?

As many other cell types, leukocytes possess several mechanisms that directly regulate the functionality of GPCRs.<sup>13,25–28</sup> These are mainly controlled by GPCR interactions with three protein families: heterotrimeric ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) G-proteins, G-protein-coupled receptor kinases (GRKs), and  $\beta$ -arrestins. Upon stimulation, a GPCR functions as a guanine nucleotide exchange factor that catalyzes the exchange of GTP for GDP on  $G\alpha$  subunits of the heterotrimeric G-protein. In leukocytes,  $G\alpha$  subunits of the pertussis toxin-sensitive  $G_i$  subfamily play critical roles for the signaling of many chemokine and chemoattractant receptors. The dissociation of  $G\beta\gamma$  from  $G\alpha$ -GTP allows them both to independently activate downstream signaling pathways. Leukocyte chemotactic responses are largely controlled by the released  $G\beta\gamma$ , which activates phosphoinositide 3-kinase (PI3K) and phosphoinositide-specific phospholipase  $C\beta$  (PLC)/inositol-trisphosphate (IP3)/diacylglycerol effector pathways.<sup>29,30</sup>

The termination of GPCR signaling relies on the intrinsic GTPase activity of the  $G\alpha$  subunit that hydrolyzes GTP and reassembles as  $G\alpha$ -GDP with  $G\beta\gamma$  to form an inactive heterotrimer. This  $G\alpha$  GTPase

reaction is accelerated by a family of proteins, called “regulators of G-protein signalling” (RGS proteins) to facilitate the rapid turning off GPCR signaling. Hence, RGS proteins are considered key desensitizers of G-protein-signaling pathways.<sup>27</sup> In addition, another negative feedback loop evolved to prevent the overstimulation of cells through excessive GPCR stimulation. Following agonist binding and G-protein activation, GPCRs become rapidly phosphorylated on their cytoplasmic tails and intracellular C-termini by specific enzymes, usually members of the GRK family.<sup>31,32</sup> The pattern of receptor phosphorylation creates a barcode with high-affinity binding sites for the recruitment of  $\beta$ -arrestin proteins.<sup>33</sup> As a consequence, the increased binding of  $\beta$ -arrestin to the receptor sterically hinders the GPCR interaction with G-proteins. This uncoupling of G-proteins from the receptor, referred to as “receptor desensitization”, prevents further GPCR activation by repeated agonist stimulation over seconds to minutes.<sup>34</sup>  $\beta$ -arrestins can also control more long-lasting desensitization of GPCRs over minutes to hours. As they can interact with clathrin-coated pits, their binding to GPCRs also targets receptors for internalization. Besides, GPCR internalization can also follow other pathways (arrestin independent, clathrin independent) or not occur at all.<sup>35</sup> Depending on the intracellular trafficking route, the internalized receptors may be subject to degradation, leading to the downregulation of cell surface GPCRs. Alternatively, targeting internalized GPCR to recycling endosomes and back to the plasma membrane can quickly replenish GPCRs on the cell surface.<sup>35</sup> In addition to their important roles in receptor inactivation and internalization, GPCR-recruited  $\beta$ -arrestins also act as signaling mediators by directly coupling to numerous signaling molecules.<sup>36</sup> At many GPCRs,  $\beta$ -arrestin-mediated signaling and classical G-protein signaling can have distinct biochemical and physiological actions from one another. A relatively new paradigm on GPCR signaling considers the allosteric interaction among the ligand, the GPCR, and the transducers ( $\beta$ -arrestins and GRKs) and how their contribution influences the bias for G-protein- or  $\beta$ -arrestin-mediated signaling.<sup>28</sup> Resulting from this paradigm, the concept of biased agonism, ie, that different ligands at the same GPCR are able to activate some signaling pathways while blocking others, is of particular interest for many chemokine receptors with prominent roles in leukocyte navigation.<sup>37</sup>

In the following, we will point out the most commonly and recently discussed mechanisms, which would allow the control of leukocyte navigation. Given the before-mentioned regulation mechanisms for GPCR functionality, a “One-GPCR system” appears already sufficient to control chemokinesis and chemotaxis of leukocytes (Figure 2A–E). To increase the sensitivity for attractant gradients, leukocytes can upregulate the cell surface expression of GPCRs. Some leukocytes store GPCRs in intracellular vesicles, which can fuse with the plasma membrane under conditions of leukocyte activation.<sup>38</sup> While the release of GPCR from vesicular stores occurs within minutes to hours, most leukocytes often respond to external stimuli by changing their cell surface GPCR profile over hours to days. These changes are primarily based on the induction of gene-transcription networks, which also include GPCR proteins, when leukocytes transition between cell states

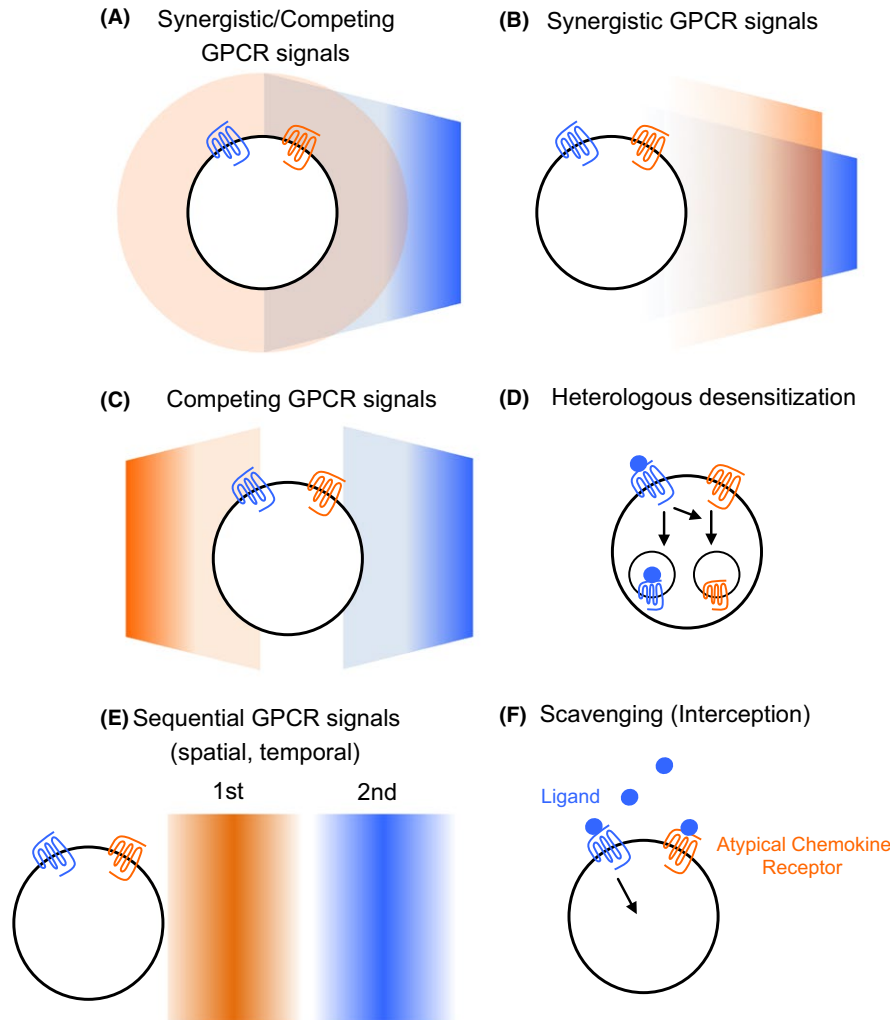


**FIGURE 2** Regulation of G-protein-coupled receptor (GPCR) functionality in “One-GPCR systems”. (A) To increase responsiveness to a chemoattractant cue, leukocytes can upregulate the number of cell surface GPCR. This can occur rapidly by fusion of GPCR-containing vesicles with the plasma membrane (left) or with temporal delay by transcriptional control of GPCR expression (right). (B) The formation of homomers (dimers, oligomers, and multimers) can up- or downregulate the responsiveness to GPCR signals. (C) Receptor antagonists or partial agonists that bind to the same GPCR as the agonist can block or dampen promigratory signaling, respectively. (D) Agonist-induced receptor desensitization leads to G-protein uncoupling or GPCR internalization, and thus attenuates the signaling responsiveness of the GPCR. Depending on the intracellular trafficking pathway of the internalized loaded GPCR, receptors are either degraded or recycled back to the plasma membrane in their unloaded form. (E) Binding of more than one agonist can lead to ligand-biased GPCR signaling and the induction of distinct signaling pathways. Alternatively, these distinct signals could be sequentially sensed along a leukocyte migration path

or undergo differentiation (Figure 2A). Multimerization of GPCRs, including homomerization of the same GPCR type, provides another level of GPCR regulation.<sup>25</sup> A recent study highlighted the functional importance of GPCR oligomerization in promoting leukocyte chemotaxis (Figure 2B). By lowering cellular cholesterol, inflammatory stimuli enabled GPCR oligomers that acted as scaffolds for distinct promigratory signaling pathways to foster efficient leukocyte migration.<sup>39</sup> However, GPCR multimerization has also been reported to dampen GPCR responsiveness.<sup>25</sup> Several other mechanisms can attenuate the sensitivity of leukocytes for chemokines and chemoattractants. One involves the presence of a receptor antagonist that binds to the same GPCR as the gradient-forming attractant agonist, but exerts blocking function for promigratory signaling (Figure 2C). More cell-intrinsic mechanisms include the downregulation of GPCR expression by lowering GPCR mRNA stability<sup>40</sup> or enhancing GPCR degradation.<sup>41</sup> As discussed above, agonist-induced receptor desensitization also attenuates the signaling responsiveness of GPCRs. Upon homologous desensitization, when GRKs phosphorylate agonist-specific GPCRs, the further recruitment of  $\beta$ -arrestins can lead to G-protein uncoupling and internalization of GPCRs (Figure 2D). This process explains why many leukocyte chemotaxis responses toward single attractants are dose dependent and follow a bell-shaped curve,

with higher concentrations resulting in stasis.<sup>42</sup> Homologous desensitization may be particularly relevant when leukocytes sense high attractant concentration in situ, eg, at the center of an attractant source.<sup>43</sup> Under such conditions, the majority of cell surface GPCRs is likely to undergo desensitization, internalization, and degradation, which may then result in cellular unresponsiveness to continuous or repeated stimulation through the same attractant. As a strategy to remain responsive to subsequent stimulation in gradients, some leukocytes internalize GPCRs, deploy its ligand cargo into lysosomes, and quickly recycle unloaded GPCRs back to the plasma membrane.<sup>44</sup> Thus, by eliminating chemotactic factors from the extracellular environments, classic GPCRs can acquire additional scavenging function. This “consumption” of external attractant may also impact the chemotactic milieu by reducing the strength of attractant cues.<sup>44</sup> Instead of only binding one agonist, many “One-GPCR systems” can bind and respond to two or more chemoattractants and activate downstream signaling (Figure 2E). Sensing these distinct attractants through the same GPCR in a spatial or temporal sequence can provide another navigation strategy.

In our body, leukocytes are rarely exposed to only one chemotactic factor. For efficient trafficking between organs and coordinated movement within tissues, most leukocytes express more than one GPCR on their surface and sense a wide range of chemokines

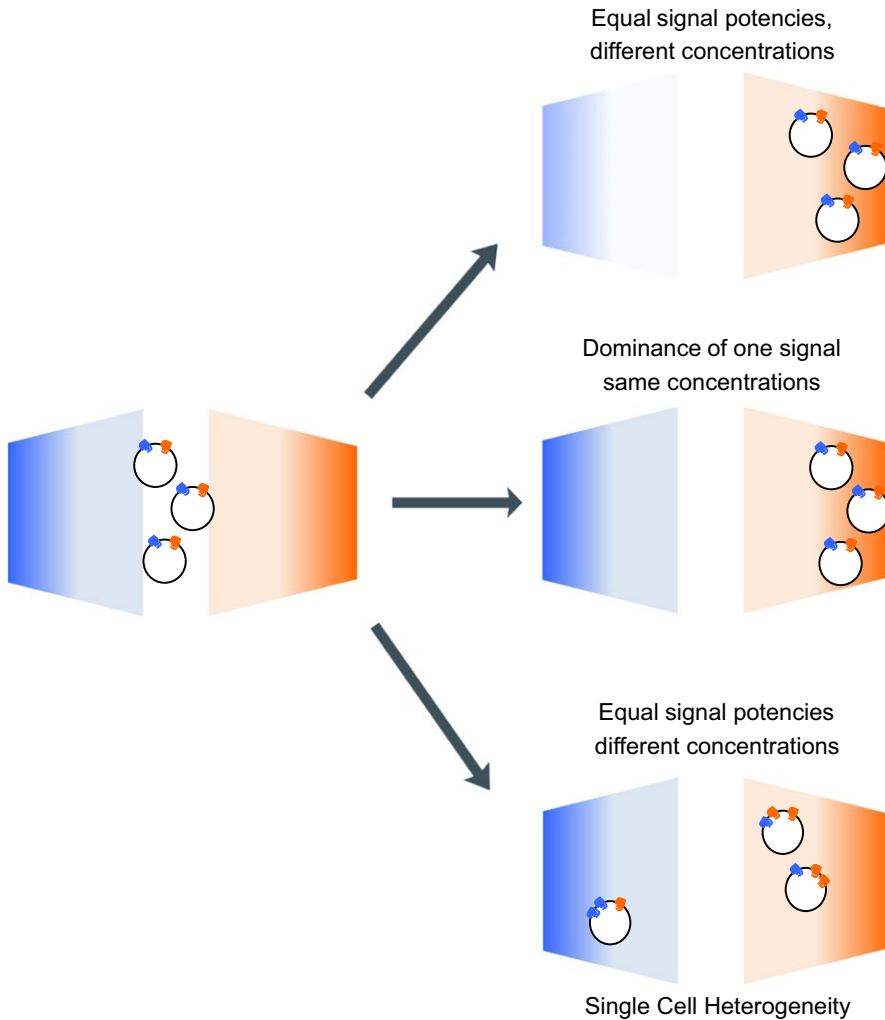


**FIGURE 3** Navigation strategies of leukocytes expressing two distinct G-protein-coupled receptor (GPCR) types (“Two-GPCR systems”) in complex chemoattractant fields. (A) Leukocytes exposed to a homogenous field of attractant 1 encounter a second-graded signal from attractant 2 that acts “on top”. (B) Leukocytes sense two attractant gradients that are oriented in the same direction and overlap, usually resulting in additive effects. (C) When leukocytes encounter two spatially opposing attractant gradients, the two GPCR signals may compete for guiding leukocytes into one or the other direction. Several factors determine the outcome of the directed migration response, some of which are depicted in Fig. 4 or discussed in the main text. (D) Agonist-independent heterologous receptor desensitization provides one form of GPCR crosstalk that leads to the dominance of one GPCR signal over others. (E) Step-by-step navigation through sequential chemotactic fields. When the two signals are spatially segregated, leukocytes first respond to signal 1 only, before maneuvering itself into a competitive situation with a second signal, as in (C). (F) Two-GPCR systems also include leukocytes co-expressing a signaling GPCR and non-signaling, scavenging GPCR for fine modulating the chemotactic response to one attractant agonist

and chemoattractants. Directional decision-making in lymphoid and inflamed organs requires leukocytes to constantly sense, compare, and integrate numerous GPCR signals emanating from multiple sources. To efficiently navigate in a complex milieu of attractant gradients, leukocytes need to respond to combinational signals and prioritize between competing signals. While this aspect of GPCR signal integration has already been of interest decades ago, it has regained momentum with the recent developments in fabricating microfluidic-based chemotaxis devices. We will now briefly summarize common mechanisms and concepts for leukocyte navigation, which become already relevant in the presence of a second GPCR signaling system (“Two-GPCR systems”) (Figure 3). As leukocytes

often use combinations of more than two-GPCR systems, these principles could be further expanded.

Manifold scenarios of attractant interpretation are conceivable, when leukocytes sense two chemoattractant signals through two different GPCR types (Figure 3). The outcome for leukocyte guidance depends on several determining parameters: (a) the spatial distribution of each signal (uniform, graded), (b) the spatial orientation of both signals toward each other (same or opposing directions), and (c) the ratio of signaling input that leukocytes receive from both signals (equal, unequal). The latter point includes both cell-intrinsic features and cell-extrinsic conditions. Unequal signaling input can result from different cell surface expression of the two-GPCR types



**FIGURE 4** Prioritizing G-protein-coupled receptor (GPCR) signals in opposing attractant gradients. Leukocytes exposed to two opposing signals with the same chemoattractive potencies will likely find migratory equilibrium in the middle of both gradients (Fig. 3C). Prioritization of one signal over the other can have several causes, with some examples depicted here: (A) Lower concentrations of attractant 1 lead to chemotaxis toward higher concentrations of attractant 2, given that both signals have the same chemoattractive potencies. (B) At the same concentration levels, one signal is dominant over the other, eg, due to heterologous desensitization or signaling hierarchies. (C) Single cell heterogeneity on the basis of cell surface expression of both GPCR types causes diverse single cell migration responses in a leukocyte population

or different strength of the downstream signaling pathway. In both cases, one chemoattractant signal dominates over the other. This can also occur in leukocytes with equal signaling input from both GPCR systems under conditions of unequal concentrations of both signals in the extracellular space. Many of these scenarios have already been addressed with select leukocyte subsets and their cell-specific GPCR combinations in controlled *in vitro* microenvironments, and examples will be discussed in the next paragraphs about specific leukocyte subsets (neutrophils, T cell, and DCs). As discussed before, homogenous chemoattractant fields can induce leukocyte polarity and chemokinesis. A second-graded signal “on top” can then redirect prepolarized leukocytes and guide them toward other locations. However, if the uniform attractant field is very potent, it will retain leukocytes and attenuate chemotaxis toward the second-graded signal (Figure 3A).<sup>45–48</sup> Under conditions when both attractant signals are oriented in the same direction and overlap, the two-GPCR signals have usually additive effects for leukocyte chemotaxis (Figure 3B). In the case of promigratory signals, chemotactic factors act often synergistically to improve chemotaxis along the same direction.<sup>49</sup> Physiologically, this might be of particular relevance for tissue sites where overlapping attractant gradients originate from the same local area. A special variant

of GPCR signal synergism has initially been observed in migrating human and mouse neutrophil populations *in vitro*. During their chemotaxis in response to shallow N-formyl-peptides (fMLF) gradients, neutrophils released the lipid leukotriene B<sub>4</sub> (LTB<sub>4</sub>) that acted on top of the early chemotactic factor as a signal relay molecule to improve chemotaxis of the whole neutrophil population.<sup>50</sup> Under conditions when leukocytes encounter two spatially opposing gradients, the two-GPCR signals compete for guiding leukocytes into one or the other direction (Figures 3C and 4). Leukocytes exposed to two opposing attractants with equal signaling strength will likely accumulate and find migratory equilibrium in the middle of both gradients (Figure 3C).<sup>47,48,51,52</sup> However, an unequal signaling input from both signals favors the movement of leukocytes into one direction (Figure 4A).<sup>47</sup> It is known for decades that specific GPCR signals can dominate over other GPCR signals, which enables leukocytes to prioritize some chemoattractant signals over others (Figure 4B).<sup>42,45</sup> This aspect of GPCR crosstalk provided a first conceptual framework for neutrophils traveling in a stepwise fashion from the blood into infected tissues.<sup>45</sup> Initially, it was discussed that heterologous desensitization of GPCRs might be an underlying mechanism for prioritizing chemotactic cues. In contrast to homologous desensitization (see above), heterologous GPCR



desensitization is agonist independent and results from the phosphorylation of one GPCR by a kinase activated through the signaling cascade of another activated GPCR (Figure 3D). Thus, one-GPCR signal can act as desensitizing signal for multiple other GPCR types, causing leukocytes to become refractory to several chemotactic stimuli within minutes. This rapid cross-phosphorylation has been linked to second-messenger kinases, particularly protein kinase C, which phosphorylates the GPCR at sites different from GRKs.<sup>53</sup> For neutrophil navigation in opposing gradients, it was later proposed that the dominance of certain GPCR signals is regulated by events downstream of the receptors. According to this concept, referred to as “signaling hierarchy”, special GPCRs induce intracellular signaling pathways that can override signaling pathways downstream of other GPCR signals<sup>42,54</sup> (see below for details). Leukocyte populations in tissues rarely consist of identical cell clones, but are rather characterized by many cells with slightly heterogeneous expression of GPCR combinations. Thus, single-cell heterogeneity on the basis of combinatorial GPCR expression may influence the population response in two opposing attractants with equal signaling strength (Figure 4C). In many physiological settings, leukocytes are thought to navigate step-by-step through sequential chemotactic fields. Under conditions of two spatially segregated chemotactic signals in sequence (Figure 3E), leukocytes may first move along a first attractant gradient (Figure 1A), and then subsequently sense the gradient of a second, more distant attractant. During the transition from first to second attractant gradient leukocytes then encounter a competitive situation between two gradients (Figure 3C). When the second signal is dominant over the first one, it is plausible that leukocytes are recruited to and then retained at the distant site. Importantly, even without any dominance between the signals, leukocytes can migrate away from a first attractant and travel toward a second, more distant attractant.<sup>45</sup> In principle, this also allows leukocytes to leave chemotactic fields of high, saturating concentrations. Moreover, leukocytes remain responsive for additional attractant changes, while moving back and forth in the equilibrium between both gradients. Thus, interpreting two distinct signals in sequence allows efficient navigation patterns that are not possible with only one attractant presented in the same spatial array. We want to point out that the sequential sensing of attractant gradients can also be temporally controlled (Figure 3E). Leukocytes may undergo sensing of a first attractant gradient, while a second attractant arises newly in close vicinity. In the course of an immune response in lymphoid or inflamed tissues, this may result from chemoattractant secretion of nearby activated tissue-resident cells or recently recruited, blood-derived leukocytes. As most immune responses are choreographed by a sequence of timely separated cellular events, it is likely that this aspect of sequential sensing has strong influence on leukocyte navigation in situ. In our contemplation on leukocyte navigation by “Two-GPCR systems”, we have focused until now on GPCRs with signaling function. However, many leukocyte subsets additionally express members of the atypical chemokine receptor (ACKR) family on their surface. Also referred to as scavenging receptors, ACKRs are GPCRs with promiscuous

binding affinity for multiple chemokines. As they do not couple to G-proteins, they are unable to induce intracellular signaling pathways. Instead, ACKRs can actively internalize and target chemokines for lysosomal degradation.<sup>55</sup> Functional co-expression of conventional chemokine receptors and ACKRs in leukocytes has been implicated in the fine-tuning of leukocyte chemotaxis, with ACKRs acting as rheostats in restricting excessive migration in response to moderate chemokine levels (Figure 3F).<sup>56-58</sup>

As we have just outlined, leukocytes have evolved several molecular mechanisms that allow an enormous spectrum of GPCR-controlled navigation strategies. Many of the emerging concepts for leukocyte navigation in complex chemoattractant fields have been developed in controlled *in vitro* systems, some of which by studying only one select leukocyte subset. Rigorous testing and proving the relevance of these concepts for leukocyte migration in the complexity of mammalian organs and tissues is technically very challenging. However, systematic studies in mice have started to delineate GPCR-controlled navigation strategies and their influence on physiological trafficking patterns through lymphoid organs and inflamed peripheral tissues. In the following, we will discuss our current knowledge on the role of GPCR signals for the migration patterns of three leukocyte subsets (neutrophils, T cells, and DCs). Each of these immune cell types has evolved their own trafficking paths to fit their effector functions. We will highlight recent studies that conceptually advanced our understanding of GPCR-controlled leukocyte navigation in mammalian tissues.

## 2 | NEUTROPHILS

Neutrophils are pivotal effector cells of our innate immune response and the first line of defense for eliminating bacteria and fungi in tissues.<sup>59</sup> Recent studies also implicate regulatory functions of neutrophils in tissue homeostasis and cancer.<sup>60</sup> In mammals, they are produced in the bone marrow and are continuously released into the bloodstream to patrol the intravascular compartment under homeostasis. Neutrophils circulate in the blood for ca. 12 hours under the influence of circadian rhythms, before they are mostly cleared into the bone marrow, but also other extramedullary tissues.<sup>61</sup> In contrast to the general belief that naive peripheral tissues are free of neutrophils, recent work suggests that low numbers of neutrophils are present in many tissues under steady state.<sup>62</sup> Upon inflammation and infection, the release of neutrophils from bone marrow increases their concentration in the blood by several folds.<sup>63</sup> Moreover, large numbers of circulating neutrophils then infiltrate inflamed tissues where they sense activated vascular endothelium. In agreement with their critical role as first responders, neutrophils have evolved robust strategies to infiltrate inflamed tissues extremely fast and orient themselves to detect potential sites of microbial invasion with great precision. In many organs, the exit from the vascular compartment involves a sequence of exquisitely coordinated processes: (a) intravascular arrest, migration, and crawling along the activated endothelial cells, and (b) breaching multiple layers of the vascular wall

(venular endothelium, venular basement membrane, and pericytes).<sup>64</sup> Once neutrophils have traversed this barrier, they are primed for the rapid detection of damaged tissue or microbes in the extravascular space. At sites of cell death in the interstitial space, neutrophils undergo sequential phases of highly coordinated chemotaxis and cell cluster formation, now commonly referred to as neutrophil swarming.<sup>65,66</sup> As true sentinel cells, they sense almost any form of tissue insult, rapidly localize it in any inflamed organ and efficiently move toward the core of the inflammation or infection site. Neutrophils have been shown to express on their cell surface more than 30 GPCR types, which can all bind chemotactic molecules. Accordingly, neutrophils are responsive to a broad array of chemically diverse chemoattractants, including chemokines, lipids, complement factors, formylated peptides, nucleotides, alarmins, and proteolytic (ECM) fragments.<sup>67-69</sup> Many of these chemotactic factors are usually present at sites of *in vivo* inflammation. How the crosstalk between these GPCR signals guides neutrophil recruitment in inflamed tissues has been of huge interest since decades.

Early *in vitro* work with human polymorphonuclear neutrophils (PMN) characterized the strength of chemically distinct chemoattractant classes to desensitize calcium-ion mobilization, as a read-out of GPCR downstream signaling, to one another (Figures 2D and 3D).<sup>53,70</sup> Peptide chemoattractants, including N-formylmethionyl-leucyl-phenylalanine (fMLF), the complement factor C5a, and the chemokine CXCL8 (IL-8), cross-desensitized one another to different extents (fMLF > C5a > IL-8). Moreover, peptide chemoattractants, in particular fMLF, cross-desensitized lipid chemoattractants, including platelet-activating factor (PAF) and LTB4. In contrast, lipid and purinergic agonists failed to desensitize to the peptide chemoattractants.<sup>70</sup> These results were extended to neutrophil chemotaxis in the presence of multiple chemoattractants, showing a hierarchy of resistance to competing signals, with fMLF and C5a inhibiting migration to other chemoattractants (Figure 3D).<sup>71,72</sup> Elegant under-agarose chemotaxis experiments proposed the concept that neutrophils can navigate through complex chemoattractant fields by migrating in a step-by-step manner in response to one agonist source after another. Thus, their ultimate positions are determined combinatorially by the array of GPCRs they express and by the sequence of chemoattractant gradients they encounter (Figure 3E).<sup>45</sup> In this concept, pathogen-derived "end-target" chemoattractants (N-formyl peptides, C5a) will dominate over host tissue-derived "intermediate-target" chemotactic factors (CXCL8, LTB4) in their ability to guide neutrophil migration (Figure 3A, C, E).<sup>45</sup> This dominance of end-target attractants has been suggested to depend on the activation of the p38 MAPK pathway, which can override the phosphoinositide 3-kinase (PI3K) pathway activated by intermediate-target attractants.<sup>42,54</sup> Importantly, neutrophil navigation is also possible in a spatially sequential array of intermediate-target chemoattractants (Figure 3E).<sup>45,47,51,73</sup> Another aspect of crosstalk between end-target and intermediate-target chemoattractants has not been considered in these early studies, but is also functionally relevant for neutrophil navigation. Already small amounts of fMLF or C5a can stimulate neutrophils to secrete intermediate-target attractants, in particular

LTB4.<sup>50,74</sup> This paracrine signaling mechanism becomes very relevant in chemotaxing neutrophil populations. Neutrophil-released LTB4 functions as a signal relay molecule and amplifies in a feed-forward manner the chemotactic response to subsaturating concentrations of end-target attractants (Figure 3B).<sup>50,75</sup> Recent developments in microfabricated devices and microfluidic-based chemotaxis systems now allow a more detailed analysis of neutrophil migration responses in complex chemoattractant milieus, providing deeper insight into neutrophil navigation in opposing gradients (Figure 3C),<sup>47,73,76-78</sup> synergistic gradients (Figure 3B),<sup>49</sup> and geometrically complex environments.<sup>49,77</sup> Moreover, even the analysis of GPCR signaling underlying neutrophil swarming dynamics is now possible due to novel micropatterning techniques.<sup>75</sup> As human PMNs, mouse neutrophils express a range of functional GPCRs to sense N-formyl peptides (through FPR1 and FPR2), C5a (mainly through C5ar1), LTB4 (mainly through LTB4R1/BLT1), and several chemokines including CXCL1, CXCL2, CXCL5 (through CXCR2), CXCL12 (through CXCR4), and several CC-chemokines (through CCR1).<sup>67</sup> Some of these GPCRs are present in secretory vesicles that can rapidly fuse with the plasma membrane upon neutrophil activation, allowing the cells to increase their responsiveness to chemotactic cues (Figure 2A).<sup>38</sup> Other reported mechanisms to control GPCR functionality in neutrophils include competitive antagonism or partial agonism (Figure 2C),<sup>79</sup> receptor multimerization (Figure 2B), differential agonist responses (Figure 2E),<sup>80</sup> and agonist scavenging through simultaneous expression of ACKRs (Figure 3F).<sup>56</sup> Homologous desensitization is a well-known phenomenon for almost all neutrophil-expressed GPCRs (Figure 2D). While GPCR transducers (G-protein subunit types, GRKs, and arrestins) and RGS proteins have been linked to the function of some GPCRs in primary mouse neutrophils,<sup>32,81-88</sup> we are still missing a detailed understanding of their exact contributions to neutrophil navigation in complex chemoattractant fields.

Clearly, neutrophils are equipped with many GPCR types and mechanisms to dynamically control GPCR activity, allowing them to use various navigation strategies in the presence of multiple chemotactic cues. Moreover, neutrophils have been shown to perform fugetaxis against the direction of a gradient of high concentration chemokine, a process that is mechanistically not well understood (Figure 1C).<sup>89</sup> However, there are still many open questions of how these mechanisms contribute to the neutrophil migration patterns observed *in situ*. Intravital microscopy studies of GPCR-deficient neutrophils provided novel insight into how GPCR signals choreograph the physiological trafficking paths of neutrophils. In the following, we will point out that GPCR signals are important throughout many steps in the lifecycle of a neutrophil. In particular, we will highlight studies that advanced our concepts of GPCR-controlled neutrophil navigation in mouse tissues.

## 2.1 | Bone marrow release: inhibiting chemokine-mediated retention

The majority of mature neutrophils in the body reside in the bone marrow, with an estimated ratio of 300:1 between neutrophils in the bone marrow and blood. Neutrophil release into the blood



is controlled by the interplay of retention and mobilization signals, provided by two antagonistically operating chemokine systems (Figure 3A,C). During homeostasis, the chemokine CXCL12 is produced in the bone marrow and essential for the retention of mature neutrophils that express the corresponding chemokine receptor CXCR4.<sup>90</sup> CXCL12 is expressed at highest levels by "CXCL12-abundant reticular cells" (CAR cells), but also by other stromal cells of the bone marrow, including osteoblasts.<sup>63,91</sup> The expression levels of CXCL12 in stromal cells follow a circadian rhythm, controlled by the sympathetic nervous system.<sup>92</sup> When neutrophils were freshly isolated from the bone marrow, they expressed only low levels of cell surface CXCR4, but contained high intracellular levels of CXCR4.<sup>90,93</sup> After placing these cells in medium lacking CXCL12, they upregulated cell surface CXCR4 within hours.<sup>90,93</sup> These findings suggested receptor desensitization and internalization in response to constitutively high local concentrations of CXCL12 in the bone marrow microenvironment (Figure 2D). Moreover, these data underlined that neutrophils receive constant CXCL12 signals, which position them in the bone marrow, probably by activating integrin-mediated adhesion to stromal cells.<sup>94</sup> While high concentrations of CXCL12 retain neutrophils in the bone marrow via CXCR4 signaling, low concentrations of CXCR2 ligands expressed on endothelial cells and produced by megakaryocytes facilitate neutrophil egress via CXCR2.<sup>63</sup> Thus, CXCR2 signals recruit neutrophils out of the retentive CXCL12 field and direct them to the endothelial sinus walls for entry into the blood circulation (Figure 3A). This intricately balanced interplay between retention and mobilization signals can be shifted in both directions. G-CSF treatment or acute inflammation, which increases blood serum levels of G-CSF and CXCR2 ligands, shifts the balance toward neutrophil mobilization from the bone marrow.<sup>95-98</sup> Several modes of action have been proposed for G-CSF, either leading to the lowering of CXCL12 levels in the bone marrow or triggering the release of CXCR2 ligands from endothelial cells or megakaryocytes (summarized in<sup>63</sup>). The same shift toward neutrophil egress can be achieved by conditional gene depletions of CXCR4 or CXCL12 in mice.<sup>99,100</sup> The administration of the CXCR4 antagonist AMD3100 also results in a threefold increase in the number of circulating neutrophils and a corresponding decrease in bone marrow numbers.<sup>93,101</sup> Originally, this drug effect was interpreted as direct antagonism of CXCR4 expressed by neutrophils, thereby disrupting the CXCL12-CXCR4 axis in the bone marrow (Figure 2C).<sup>90</sup> Surprisingly, subsequent studies found that AMD3100 also acted on CXCR4 expressed by bone marrow endothelial cells, and inhibited the translocation of CXCL12 across bone marrow endothelial cells, suggesting a rapid rise of CXCL12 in the blood and reversal of the CXCL12 gradient (Figure 3B).<sup>102,103</sup> These data were questioned by intravital imaging studies that could not observe clear neutrophil egress from the bone marrow upon AMD3100 treatment. Instead, the increase in circulating neutrophils through AMD3100 was explained by the release of marginated neutrophils from the lung microvasculature.<sup>104</sup> Moreover, AMD3100 had a blocking effect on the trafficking of aged blood neutrophils, which upregulate CXCR4 expression over time, enabling them to home back to the bone marrow

for clearance.<sup>104</sup> The exact mode of action of AMD3100 is still controversially discussed.<sup>90</sup>

Insight into the physiological consequences of shifting the chemokine balance in the opposite direction, toward retention in the bone marrow, comes from an extremely rare congenital human immunodeficiency disease. Warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome is an autosomal dominant human disorder caused by gain-of-function mutations in the C-tail of CXCR4.<sup>105,106</sup> WHIM syndrome involves myelokathexis, a condition characterized by the retention of mature neutrophils in the bone marrow and peripheral neutropenia. WHIM-associated truncations or mutations in the carboxy-terminal tail of CXCR4 result in the loss of multiple serine residues, which are target sites for GRK-mediated receptor phosphorylation. Truncated forms of CXCR4 are impaired in agonist-induced homologous desensitization and internalization, and cause enhanced signaling upon CXCL12 stimulation.<sup>106</sup> This leads to stronger anchorage of mature neutrophils in the bone marrow, counteracting CXCR2-mediated chemotaxis toward vessels and neutrophil egress. However, administration of G-CSF or ADM3100 to WHIM patients can overcome this imbalance and cause an increase in circulating neutrophil numbers.<sup>106</sup>

## 2.2 | Getting to the site of inflammation: navigation along multiple chemokine fields

Blood-circulating neutrophils detect sites of tissue injury and infection by interacting with the luminal surface of activated venular endothelium. In response to tissue-released inflammatory cytokines, endothelial cells upregulate P-selectin, E-selectin, and integrin ligands, such as ICAM-1 and VCAM-1, to promote interactions between neutrophils and the vessel wall. This induces neutrophils to undergo a multistep adhesion cascade along the endothelial lining before emigrating from the vessel into the inflamed tissue interstitium.<sup>107</sup> Chemokines are present on the luminal surface of endothelial cells and cause arrest of rolling neutrophils by triggering the activation of integrins. Many studies showed an involvement of CXCR2 ligands, in particular CXCL1, but also CXCL2 and CXCL5, in this process.<sup>108,109</sup> These chemokines are primarily expressed and released by endothelial cells,<sup>110,111</sup> but also tissue-resident cells, including pericytes, perivascular macrophages, and mast cells, can act as important chemokine sources.<sup>112-115</sup> Due to their high-affinity binding sites for heparin sulfate (HS) GAG most chemokines become immobilized by HS GAG on the luminal side of the endothelium,<sup>116</sup> and defects in HS GAG synthesis have been shown to decrease immobilization of chemokines and neutrophil transmigration into inflamed tissues.<sup>117,118</sup> While the involvement of chemokines for neutrophil transmigration is widely established, there has been ongoing debate whether and how leukocytes sense stable chemokine gradients across the vessel wall on their way into the tissue (Figure 1B). There are ample references to support the common belief that chemotactic gradients between the apical and basolateral part of postcapillary venules promote diapedesis, ie, the outward passage of neutrophils through the vessel wall. Immunofluorescence stainings of HS GAG

moieties revealed steep gradient of HS GAG scaffolds between the luminal and abluminal aspect of skin postcapillary venules.<sup>119</sup> Under inflammatory conditions, HS GAG distribution became even more polarized by massive depositions of HS and laminin in the basolateral basement membrane of skin vessels.<sup>119</sup> Given the GAG-binding properties of many chemokines, it was proposed that these sharp HS GAG scaffold gradients might favor chemokine gradients across the venular wall. In agreement with this idea, excessive deposition of the GAG-binding chemokine CXCL1 on the apical part of inflamed blood vessels resulted in decreased neutrophil diapedesis.<sup>120</sup> Other supporting data come from experiments in which an extravascular source of CXCR2 ligands was placed in form of a gel on one side of a blood vessel.<sup>121</sup> Under these conditions, neutrophil crawling was directed to emigrate preferentially from that side of the vessel. HS GAG on the surface of the endothelium was required to present CXCL2, forming a chemotactic gradient that was followed by neutrophils. This sequestration of endothelial chemokine occurred exclusively in venules, but not other blood vessel types.<sup>121</sup> HS GAG, together with the atypical receptor ACKR1, was also discussed to establish gradients by transcytosis of chemokines.<sup>118,122,123</sup> Transcytosis involves the internalization and transport of tissue-derived inflammatory chemokines from the basal surface onto the luminal surface of endothelial cells. ACKR1 is specifically expressed by postcapillary venules and was shown to support promigratory chemokine patterns across the vessel wall for optimal leukocyte migration across the vessel wall.<sup>123,124</sup> Unlike other ACKR, ACKR1 does not act as classical scavenger receptor, and was initially discussed to promote the transcytosis of tissue-derived inflammatory chemokines from perivascular pools to the apical surface of venules.<sup>122,123</sup> Novel insight into the functional role of ACKR1 and the directional cues that guide neutrophils during diapedesis come from recent elegant experiments of Nourshargh and colleagues.<sup>125</sup> By studying TNF-induced neutrophil emigration in the inflamed mouse cremaster muscle, it was shown that neutrophils encounter the two CXCR2 ligands CXCL1 and CXCL2 in a sequential manner during their passage through the vessel wall. In a three-step process, neutrophils first sense CXCL1 produced by TNF-stimulated endothelial cells on the luminal surface of venules, promoting crawling along the endothelium. Next, neutrophils encounter CXCL2 that is retained at endothelial cell junctions by ACKR1. Notably, CXCL2 is secreted by neutrophils themselves and then presented by ACKR1, which is enriched in endothelial junctions. This self-guided migration response of neutrophils was critical for breaching of endothelial junctions and unidirectional transendothelial migration. Lastly, CXCL1 expressed by pericytes governed neutrophil crawling in the subendothelial space.<sup>125</sup> While earlier work indicated relatively restricted expression of CXCL1 to vascular and tissue-resident cells, and CXCL2 with activated neutrophils,<sup>126</sup> these novel findings highlight unexpected non-redundant roles for CXCL1 and CXCL2 to guide neutrophils through venular walls as governed by their distinct cellular sources.<sup>125</sup> Having identified this critical spatiotemporal sequence of chemokine-guided steps across the vessel wall (Figure 3E), it will be of future interest to understand how other tissue-derived attractant signals may act on top of this

and support further guidance into the underlying inflamed interstitium. As an example, neutrophils entering an inflamed peritoneum were suggested to encounter two sequential gradients in a step-by-step manner (Figure 3E).<sup>114</sup> In a lipopolysaccharide-induced peritonitis model, initial recruitment of neutrophils out of the blood vessel into the peritoneal tissue was regulated by rapid secretion of CXCL1 and CXCL2 from perivascular mast cells. Further guidance of extravasated neutrophils through the interstitium and into the peritoneal cavity depended on CXCL1 and CXCL2 produced by tissue-resident interstitial macrophages.<sup>114</sup>

### 2.3 | Getting to the site of inflammation: interpreting temporal cascades of chemically distinct chemoattractants

Most of our discussions until now focused on the role of chemokines for neutrophil guidance in the tissue, but also putative non-GAG-binding, soluble chemoattractants including C5a, LTB<sub>4</sub>, and formylated peptides are sensed by neutrophils and relevant for special types of inflammatory reactions. A mouse model of autoimmune rheumatoid arthritis, the K/BxN serum transfer model, has provided unprecedented insight into how four different chemoattractant receptors, C5aR1, LTB<sub>4</sub>R1, CCR1, and CXCR2, collaborate to recruit neutrophils into the inflamed tissue.<sup>127</sup> In this model, the inflammatory response is driven by immunoglobulin G autoantibodies to the ubiquitous glycolytic enzyme glucose-6-phosphate isomerase (GPI) leading to the formation of immune complexes that drive immune cell activation. Neutrophil infiltration into the joint is critical for the clinical signs of arthritis in this model. Several consecutive studies have elucidated a temporal cascade in which neutrophils encounter complement, lipid, and chemokine signals to enter the joint and contribute to disease progression (Figure 3E).<sup>74,128-131</sup> While it has long been known that immune complexes lead to the activation of the alternative complement system in arthritis,<sup>132,133</sup> it has only recently been found that C5aR signaling in circulating neutrophils is the key initiator for a cascade of events that ignite inflammation.<sup>131</sup> The deposition of immune complexes in the joint space induces the generation of C5a, which unexpectedly binds to HS GAG on the synovial endothelium. Neutrophils sense C5a on the joint endothelium, induce integrin-dependent arrest, and facilitate crawling along the vasculature. Subsequently, C5a signals stimulate neutrophils to secrete LTB<sub>4</sub>, which induces the entry of the first neutrophils into the joint and initiates the onset of disease.<sup>74,128,129,131</sup> After disease onset, recruited neutrophils in the joint secrete IL-1 $\beta$ , which in turn stimulates fibroblast-like synoviocytes to produce CCR1 ligands at first and later CXCR2 ligands.<sup>130</sup> CCR1 ligands are required to recruit the next wave of neutrophils into the joint, and this recruitment of neutrophils is broadly amplified in the last step of the cascade when neutrophils themselves release CXCL2 to potentially attract large numbers of neutrophils into the joint.<sup>67,130</sup>

Thus, neutrophil entry into the inflamed joint is a prime example where neutrophil recruitment is choreographed by non-redundant roles of distinct chemoattractants that collaborate sequentially in a

temporal cascade (Figure 3E). Moreover, this process highlights the rapid production of complement and LTB<sub>4</sub> to initiate a recruitment cascade at the local site of inflammation. By contrast, chemokines act later in this cascade to amplify and prolong the inflammatory response.<sup>67</sup>

## 2.4 | At the site of inflammation: synergism through lipid-mediated amplification

Another example where complement-induced LTB<sub>4</sub> secretion rapidly shapes neutrophil migration patterns has recently been described.<sup>134</sup> In a simplified fungemia mouse model, the fungi *Candida albicans* sequestered in the pulmonary vasculature and actively recruited neutrophils. Neutrophils showed swarm-like migration patterns in response to sequestered yeast, similar to previously described swarming responses in other tissues.<sup>66</sup> A small number of lung neutrophils responded within minutes and performed intravascular chemotaxis, before larger numbers of neutrophils were recruited and formed cell clusters. The immediate capture of live *C. albicans* by neutrophils depended on complement-mediated chemotaxis.<sup>134</sup> In addition, complement activation stimulated neutrophils to secrete LTB<sub>4</sub>, which amplified the directional recruitment and clustering of neutrophils in a synergistic manner (Figure 3B). This was in agreement with a previous study highlighting the importance of LTB<sub>4</sub>-mediated signal amplification during neutrophil swarming in interstitial tissue spaces at sites of sterile skin injury and in bacteria-infected lymph nodes.<sup>135</sup> Neutrophil swarming is an essential process of the neutrophil tissue response and has been observed in diverse tissues under conditions of sterile inflammation and infection with various pathogens, including bacteria, fungi, and parasites.<sup>65,66</sup> The swarming response comprises sequential phases of highly coordinated chemotaxis followed by neutrophil accumulation and the formation of substantial neutrophil clusters, which has led to the multistep attraction model of neutrophil swarming.<sup>66</sup> While the underlying signals and molecular players for some of these steps are still unclear, we know that neutrophil-derived LTB<sub>4</sub> acted on top of early released, yet unknown, chemoattractants and mediates intercellular signal relay among neutrophils to amplify interstitial recruitment in a feed-forward manner (Figure 3B). This model was in accordance with earlier *in vitro* studies identifying LTB<sub>4</sub> acted as a signal relay molecule (see above).<sup>50</sup> As a consequence, LTB<sub>4</sub>-LTB<sub>4</sub>R1 signaling enhances the radius of neutrophil recruitment from distant tissue sites and maintains the amplified chemotactic response of the neutrophil population. Together with signaling through other GPCRs, such as CXCR2 and FPR2, LTB<sub>4</sub>R1 signaling also optimizes neutrophil clustering.<sup>135</sup> Importantly, LTB<sub>4</sub> and CXCR2 signals are also relevant for the swarming response of human neutrophils.<sup>75</sup> The formation of focalized dense neutrophil aggregates is considered beneficial for isolating sites of tissue wounding from viable surrounding tissue, containing microbial invaders and concentrating the microbicidal activity of a whole neutrophil population in one swarm center.<sup>66</sup> However, neutrophil aggregation can also lead to tissue damage and removal of other cells.<sup>135,136</sup> In the context of

the lung microvasculature, intravascular neutrophil swarms that formed in response to *C. albicans* infection lead to vessel occlusion and pulmonary hemorrhages, which were reverted in the absence of LTB<sub>4</sub>R1.<sup>134</sup> How neutrophil-derived LTB<sub>4</sub> shapes and maintains a promigratory chemotactic field *in vivo* remains unclear. LTB<sub>4</sub> is considered to have a short half-life and can be rapidly turned into LTB<sub>4</sub> metabolites, which act as natural inhibitors of LTB<sub>4</sub>-mediated responses. However, it has also been demonstrated that neutrophils release LTB<sub>4</sub> in form of exosomes, which may support more prolonged effects of LTB<sub>4</sub> gradients.<sup>137</sup> Interesting insights into this issue come from studies with diabetic mice receiving skin infections with methicillin-resistant *Staphylococcus aureus* (MRSA), which leads to large neutrophil clusters in form of abscesses.<sup>138</sup> Diabetic mice produce higher levels of LTB<sub>4</sub> in the infected skin compared to non-diabetic mice. Surprisingly, neutrophils in the skin of these mice failed to assemble into an organized abscess, as excessive LTB<sub>4</sub> levels likely disrupted chemoattractant gradients in the skin and impaired directed neutrophil chemotaxis.<sup>138</sup> These findings exemplify that the appearance or absence of neutrophil swarms depends on the specific mixture of chemoattractants in a specific inflammatory environment. Although not yet studied in detail, we know that several factors, including tissue damage size, presence of pathogens, induction of cell death, and neutrophil numbers, are important for shaping the exact neutrophil-swarm phenotype in an inflamed tissue. All these factors can potentially initiate the release of chemotactic fields that may strengthen or attenuate LTB<sub>4</sub>-mediated signal amplification *in situ*.<sup>66</sup>

## 2.5 | Getting to the site of inflammation: following hierarchies

True prioritization of chemoattractant fields has been observed for neutrophils in a model of thermal-induced liver injury.<sup>139</sup> In this model, large numbers of neutrophils are recruited over hours from the liver sinusoids to a focal site of sterile hepatic necrosis. The necrotic area releases several molecules that shape the adjacent hepatic environment and ultimately form two distinct chemotactic fields at different distances from the necrotic focus. During their search for damaged tissue neutrophils sequentially navigate through these two chemotactic fields. First, neutrophils follow an intravascular gradient of GAG-bound immobilized CXCL2 that supports integrin-dependent crawling toward the necrotic focus. This CXCL2 haptotactic gradient abruptly halts, approximately 150 μm proximal to the border of the necrotic tissue. Second, neutrophils at this border continue to migrate on top of platelets along non-perfused vasculature, before entering the necrotic area.<sup>139,140</sup> When neutrophils were lacking the formyl-peptide receptor FPR1, they migrated along the healthy sinusoids to the highest concentration at the end of the haptotactic CXCL2 gradient and never entered into the platelet-rich and following necrotic zone. Given the important role of FPR1 signaling during this process, mitochondrial-derived formyl peptides released from dying hepatocytes were suggested to attract neutrophils into the necrotic area. Of interest, FPR1 signaling has also been

shown to regulate neutrophil recruitment into necrotic areas of hepatic ischemia-reperfusion injury.<sup>141</sup> In contrast to formyl peptides released from bacteria, endogenous formyl peptides are produced by mitochondria and become released upon cellular damage.<sup>142</sup> Similar to bacterial formyl peptides, also mitochondrial formyl peptides can promote neutrophil chemotaxis in an FPR-dependent manner.<sup>139,142</sup> Thus, neutrophil navigation in the liver injury model follows a spatial cascade of chemoattractants (Figure 3E) and appears to depend on the prioritization of an end-target attractant (eg, fMLF) over an intermediate-target attractant (eg, CXCL2) (Figure 3A). Recent elegant work by Kubes and colleagues revealed that neutrophils play a critical role in fully repairing the hepatic injury. In the course of this process, neutrophils at the border of the injury left the injury site and reentered healthy liver sinusoids.<sup>143</sup> However, it remains to be shown if GPCR-controlled mechanisms are involved in this form of reverse migration into the vasculature.

### 3 | T CELLS

T cells and B cells represent the cellular hallmark of the adaptive immune system. These lymphocytes are generally long-lived cells and are driven by GPCR-mediated guidance throughout their life cycle. Being released from the bone marrow as a common lymphoid progenitor, T-cell precursor settle in the thymus where they tread a highly regulated developmental path, guided by GPCRs. Once T cells have been fully matured and selected, they exit the thymus and commence their recirculation pattern between secondary lymphoid organs (SLO). As the T-cell receptor (TCR) repertoire needs to be highly diverse in order to raise the chance to efficiently detect foreign antigens against all kind of pathogens, the naive T-cell precursor frequency for a specific antigenic determinant is very low ( $\approx 1/10^5$ – $1/10^6$ ). Consequently, during a newly arising infection, antigen-presenting DC need to interact with millions of T cells in order to activate the few rare naive T cells that optimally react to the foreign antigenic determinant. To facilitate this process T cells need to efficiently migrate between and navigate within SLOs to optimize such DC encounters. Once T cells are activated and primed by a DC carrying and displaying the proper antigen, they differentiate and change their GPCR expression pattern in order to leave the SLO and enter infected tissues via the blood stream. Here, GPCRs pilot them toward infected cells where they execute their specific effector function in order to eliminate or at least control the microbial invasion.

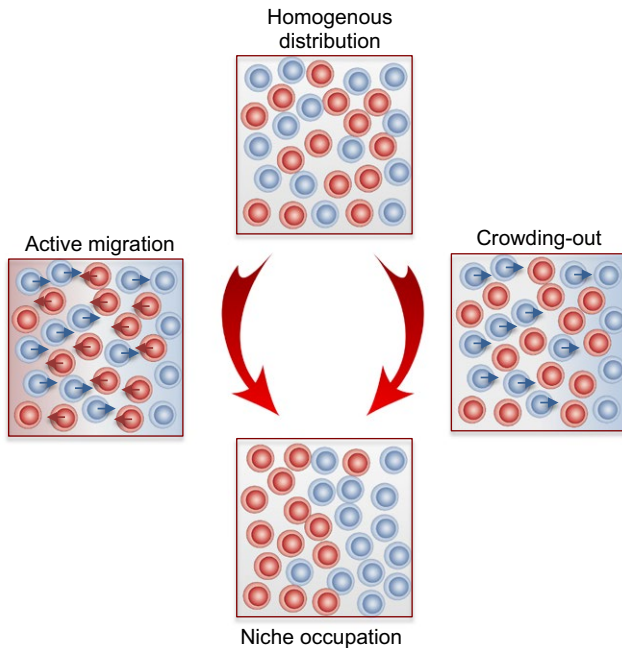
In vitro experiments to address how lymphocytes respond to chemokines were primarily based on transwell, under agarose assays or 3D ECM-like gels, showing that chemokinesis, chemotaxis, and homologous and heterologous desensitization apply to GPCR-mediated navigation of T cells.<sup>144–149</sup> However, these approaches have limitations when trying to address how T cells respond to more complex chemokine fields involving multiple GPCRs. With the development of microfluidic devices<sup>150</sup> and more recently, microfluidic mazes, T-cell exploratory patterns and chemotaxis in response to chemokines could be analyzed in more detail, while providing the

investigator with a higher level of control and precision.<sup>151</sup> Overall, however, there are only a few studies that have addressed complex GPCR interactions in T cells in vitro. In the following sections we will focus on what we have learned from in vivo experiments regarding the trafficking of T cells.

#### 3.1 | Searching for the antigen: exploration and compartmentalization

In vivo, chemo- and haptokinesis are particularly prevalent in lymphoid organs and underlie the continuous undirected or random-walk migratory activity of lymphocytes (Figure 1A).<sup>9</sup> Functionally, this migratory behavior is required to enable encounters and consecutive interactions between leukocytes. In other words it reflects the search strategy of lymphocytes to detect rare interaction partners in a large and densely populated three-dimensional space. Given the low precursor frequency of lymphocytes, such migratory behavior was predictable; yet direct visualization of lymphocyte migration by means of intravital two-photon microscopy fundamentally changed our perception how lymphocytes navigate and interact with their environment in complex tissues.<sup>8,152</sup> This migratory behavior of T cells in vivo is regulated by several factors (for review see<sup>153</sup>). (a) T cell-intrinsic molecules that regulate cellular polarization, protrusion formation, and turning patterns,<sup>154–156</sup> (b) physical constraints and guiding structures formed by stromal elements and the extracellular environment,<sup>157</sup> (c) intercellular adhesive forces<sup>158</sup>, and (d) chemokinetic cues.<sup>159</sup> The central chemokines and receptors that drive chemo-/haptokinesis of lymphocytes in lymphoid organs are CCL19/CCL21-CCR7, CXCL13-CXCR5, CXCL12-CXCR4, and LPA-LPAR2. Interestingly, these chemokines appear to primarily regulate the speed of migrating cells rather than their migratory pattern. In particular, CCR7-deficient T cells showed about 30%–50% reduced T-cell speed in LN explants as well as in situ.<sup>18,160–162</sup> Additional blockade of CXCR4 did not further decrease lymphocyte speed arguing that other factors than chemokines regulate the basal migratory speed of lymphocytes in the steady state.<sup>162,163</sup> Indeed, two recent studies provided direct evidence that LPA (lysophosphatidic acid) contributes to the migrational speed of CD4 T cells in the lymph under steady-state conditions.<sup>164,165</sup> Using intravital microscopy both studies showed that *Lpar2*-deficient CD4 T cells have a reduced migrational speed and spatial displacement as compared to wt CD4 T cells. These results add to the concept that T cells not only interpret multiple chemokines but in addition lipid signals, which together organize the localization, the migrational speed, and the search pattern of lymphocytes in SLO.

Overall, chemo- and haptokinesis are key elements of lymphocyte migration within SLO. By contrast, chemo- and haptotaxis are critical mechanisms that lymphocytes utilize in order to travel between tissues and find their specific destination within microanatomical niches (Figure 1B). In the steady state chemotactic migration of T cells typically reflects the integration of several conflicting signals detected by multiple GPCRs, like the recirculation pattern of T cells that is regulated by exit vs retention cues, as we will discuss



**FIGURE 5** Crowding out vs active migration. In a 3D space that is homogeneously populated by, eg, two different groups of cells, a redistribution and niche occupation is typically achieved by active migration toward a chemoattractant gradient by both groups of cells. However, if both cell populations have a baseline chemokinetic activity, redistribution of both populations may be based on the directional migration of only one population. The other population can passively redistributed due to space limitations (crowding-out effect)

in more detail below. In the following paragraph, we will discuss instances of chemotaxis of T cells that are dominated by single receptors assuming that potentially competing signals may be functionally negligible in these examples.

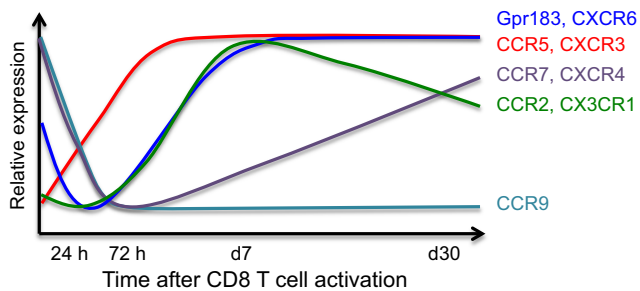
In the steady state T-cell subset distribution within the LN or the white pulp of the spleen is inhomogeneous. For example, it has been observed that the localization between CD4 and CD8 lymphocytes in the white pulp of the spleen is not fully overlapping.<sup>166</sup> Specifically, CD8 T lymphocytes populate more densely the deep paracortex, while CD4 T lymphocytes are located in slightly more peripheral areas of the white pulp or the LN paracortex, respectively. Gpr183-mediated chemotaxis is likely the basis for this differential localization. CD4 T cells express significantly higher levels of Gpr183 than CD8 T cells and the ligands for Gpr183, oxysterols, are emitted at the interfollicular area (and the bridging channel of the spleen). Reminiscent to the differential localization of T lymphocytes, oxysterols regulate the localization of cDC2 in contrast to cDC1 that are more abundant in the deep paracortex.<sup>167-169</sup> However, in principle, differential localization in the SLO may also be explained via the engagement of another GPCR in particular CCR7. The peculiarity of SLO compared to other organs is that the majority of its cellular elements (lymphocytes) are constantly in motion in a chemokinetic manner as discussed above. Therefore, in principle in a 3D-space, which is homogeneously populated by a heterogeneous group of cells,

relocalization of one group of cells could be either active or passive (Figure 5). Active migration reflects the chemotactic migration toward a newly arising chemokine gradient. Passive migration reflects a crowding-out effect of cells in a limited space by incoming cells that may themselves follow a chemotactic gradient. We have observed such a crowding-out effect in inflammatory conditions. Specifically, we compared the behavior of memory vs naive antigen-specific T cells in the context of viral infections of the LN.<sup>170</sup> In non-competitive settings, naive antigen-specific CD8 T cells rapidly (within 4-8 hours) detect and co-arrest with antigen-bearing APCs in the interfollicular area. In the presence of antigen-specific memory CD8 T cells, we observed a significant decrease in naive antigen-specific CD8 T cells numbers in the interfollicular area starting about 100 min postinfection. This coincided with a strong influx of memory CD8 T cells based on CXCR3-mediated chemotactic cues arising from the SCS area.<sup>170,171</sup> Although not directly addressed in these studies, the observed relocalization of naive CD8 T cells in the context of incoming memory CD8 T cells is well explained by a crowding-out model (Figure 5). These dramatic effects observed during inflammatory conditions may also apply in the steady state. CCR7-mediated signals support chemokinesis in the LN. However, there are indications that CCR7 also acts on a chemotactic level in the steady state guiding responding cells to the center of the LN. For example, LN-resident cDC1 that get activated at the interfollicular area migrate to the deep paracortex upon maturation and CCR7 upregulation.<sup>172</sup> Vice versa, Tfh cells that search for interacting B cells at the T/B border downregulate CCR7 to reach this area and upregulate CXCR5 to enter the B-cell follicle as a second step.<sup>173</sup> Consistently, FRC (fibroblastic reticular cells) at the T/B border produces less CCL19 than their counterparts in the paracortex.<sup>174</sup> Similarly, we observed that memory CD8 T cells express lower levels of CCR7 and are located at peripheral areas of the LN paracortex. By contrast to naive CD8 T cells that express higher levels of CCR7 and populate the deep paracortex.<sup>170</sup> So memory CD8 T cells may in part be displaced (crowding out) from the deep paracortex by naive CD8 T cells that experience a stronger chemotactic attraction to this area.

Chemotaxis not only regulates the localization of conventional T cells but also navigates NK cells and invariant T cells in SLO. Functionally, these lymphocytes are localized in closer proximity to lymphatic sinuses in order to promote a more rapid response against lymph-borne pathogens, which is essential to avoid microbial dissemination.<sup>175,176</sup> Invariant T cells are attracted to SCS via CCL20-CCR6-mediated chemotaxis.<sup>177</sup> CCL20 is produced by the lymphatic endothelium generating a gradient across the sinus floor. This gradient guides CCR6-expressing T cells toward sinus-lining macrophages that in turn provide them with IL1 and IL23 in the context of infections.

Most of the above examples identified chemotaxis indirectly, meaning by the differential localization of cells in the absence of a specific chemokine receptor. However, in the context of inflammation also direct evidence for chemotaxis of T cells has been found. Here, chemotactic signals may become dominant, both in a quantitative or qualitative manner making it easier to observe alterations





**FIGURE 6** Time-dependent changes in G-protein-coupled receptor (GPCRs) during activation of CD8 T cells. Evolution of the GPCR expression pattern of CD8 T cells after activation. The GPCR expression pattern of naive T cells (before activation) determines their search behavior for antigen-presenting DC. GPCRs expressed between d1 and d3 may help activate T cells to support their differentiation by promoting encounters with specific DC or by navigating them into distinct inflammatory environments within SLO (eg, toward licensed cDC1 that relay CD4-helper signals). GPCRs expressed during the effector phase help differentiated CD8 T cells to find target cells within infected tissues. GPCRs expressed in the memory phase determine the area of surveillance. Notably, the relative gene expression is shown on a population level, yet is highly heterogeneous or bimodal on a single cell level

in migratory behavior of T cells in a rapidly changing environment. In contrast to neutrophils or DC that show a high degree of directionality toward a chemotactic gradient, T cells rather show a meandering motion toward their target. However, occasionally T cells make directional jumps as direct evidence for chemotaxis. This has been directly visualized in the context of CCR5- and CXCR3-mediated signals.<sup>170,171,178,179</sup>

### 3.2 | Recirculation: modulation of sensitivity

Homologous receptor desensitization as a means to regulate GPCR signaling is a well-established concept *in vitro* (Figure 2D). In particular, chemokine receptor internalization on T cells has been demonstrated for CXCR4, CCR7, CCR5, and CXCR3.<sup>145-148</sup> *In vivo*, CXCR3 also gets rapidly internalized on memory CD8 T cells upon ligand exposure in the context of viral infections.<sup>171</sup> However, to what level CXCR3 desensitization modulates T-cell migration *in vivo* during inflammatory conditions and how this contributes to pathogen resistance remains largely unknown. Probably the best established example for the physiological relevance of receptor desensitization is S1PR1. S1PR1 is essential for the exit of lymphocytes from SLO and the thymus.<sup>180</sup> The ligand S1P is expressed by hematopoietic cells, in particular erythrocytes leading to high concentration in the blood. In order to generate a gradient, tissue expressed S1P lyases efficiently degrade S1P, thereby reducing its concentration in tissues. T cells express high levels of S1PR1 on their surface, but once they enter the blood stream, S1PR1 is rapidly down modulated, promoting T-cell entry into SLO.<sup>181</sup> Within SLO, S1P concentrations are low, leading to re-expression of S1PR1 on the cell surface and lymphocytes regain the ability to exit SLO over time (for review<sup>182</sup>). Added to the

cyclic surface expression pattern of S1PR1, also CCR7 surface expression appears to undergo a periodic pattern. In particular, it was found that CCL19 leads to receptor occupation and internalization of CCR7, functionally impairing its responsiveness *in vitro*.<sup>183</sup> However, in CCL19-deficient animals lymphocyte dwell times in LN appear to be unaltered, so it remains to be shown if and to what level modulation of CCR7 expression impacts on T-cell migration and in particular recirculation. By contrast, the functional relevance for CXCR4 receptor desensitization *in vivo* is more evident. In particular, a desensitization resistant gain-of-function mutation of CXCR4 (CXCR4<sup>1013</sup>) that underlies the WHIM syndrome in humans has been introduced into a mouse model.<sup>184</sup> CXCR4<sup>wt/1013</sup> heterozygous animals show reduced thymocyte and splenic T-cell counts, while absolute T-cell numbers were increased in LNs. These alterations in T lymphocyte trafficking between SLO and the blood could be normalized by CXCR4 inhibitors, underscoring the importance of CXCR4 and its desensitization for lymphocyte recirculation.

So how is GPCR desensitization regulated in T cells? Regarding S1PR1 desensitization the kinase GRK2 plays a central part.<sup>185</sup> Notably, GRK2 also regulates the desensitization of other chemokine receptors like CCR5 in T cells.<sup>186</sup> During chronic inflammatory conditions in humans, GRK2 expression levels appear to be significantly reduced on T cells supporting the notion that GRK2-mediated GPCR desensitization plays an important role in T-cell trafficking beyond recirculation. Counterintuitively, absence of GRK6, which is also highly expressed in T cells, strongly impairs CXCR4-mediated chemotaxis toward CXCL12 *in vitro*. This shows that GRKs work in a more complex fashion that likely goes beyond GPCR desensitization.<sup>187</sup>

Besides GRKs, a large group of RGS proteins is known to regulate GPCR signaling in lymphocytes.<sup>188</sup> Several RGS proteins are highly expressed in lymphocytes. However, so far only RGS1 deficiency revealed a definitive role of these proteins in lymphocyte migration *in vivo*. Specifically, RGS1 reduces the sensitivity toward CXCR4 and CCR7 ligands,<sup>189</sup> and in particular regulates the trafficking and residency of T cells in the gut.<sup>190</sup> Additionally, Trm show a significant upregulation of RGS1 compared to Tcm arguing for a more general function of this protein in regulating tissue residency of T lymphocytes.<sup>191</sup>

Changes in sensitivity toward a GPCR ligand can also be modulated by changes in GPCR expression levels (Figure 2A). In T cells dynamic changes in GPCR surface expression is tightly connected to their biological function. We will discuss this in detail below as this involves multiple GPCRs. A special case is periodic changes in GPCR expression on lymphocytes in the context of the circadian rhythm. These changes modulate the recirculation and migration pattern of lymphocytes and myeloid cells and therefore have a profound impact on both innate and adaptive immune responses.<sup>192,193</sup> This has been excellently reviewed elsewhere<sup>194</sup> and will not be further discussed here.

In summary, there is robust *in vivo* evidence for the importance of GPCR desensitization regarding T-cell recirculation, yet to what level this mechanism regulates T-cell trafficking toward inflammatory



chemokines in the context of infections still needs to be addressed in the future.

### 3.3 | Development and differentiation: it is (almost) all about time and space

Time-dependent changes in GPCR expression levels can be typically seen during development of cells and organs, but also during activation of cells in the context of inflammation, tissue damage, and infections. Time-dependent and spatial changes in GPCR signaling accompany T cells throughout their life time and help them to navigate through tissues. Within tissues GPCRs help T cells to settle in specific niches that provide further instruction signals or direct them to target areas that require their specific effector function.

Upon arrival in the thymus, T-cell progenitors undergo a highly complex instruction regimen that will allow them to express and rearrange their TCR chains. T cells with an intermediate affinity toward peptide bound MHC complexes are then further selected via processes called positive and negative selection.<sup>195</sup> Importantly, the functional development and instruction of thymocytes to mature lymphocytes is reflected by a physical journey of these cells through the thymus. During this “round-trip” thymocytes start at the cortico-medullary junction, move to the thymic cortex and the subcapsular zone and then back to the cortico-medullary junction and the thymic medulla.<sup>196</sup> From here, mature thymocytes exit the thymus via S1P-S1PR1 signals and commence their GPCR-dependent recirculation between the blood and SLO.<sup>197,198</sup> Likewise, the migration within the thymus is dependent of the timed expression of chemokine receptors in particular CXCR4, CCR7, and CCR9.<sup>196</sup> CCR7 signals contribute to thymocyte transition from the cortico-medullary junction to the outer cortex<sup>199</sup> and are critical for their migration back to the medulla.<sup>200</sup> Specifically CCL21Ser (and not CCL21Leu or CCL19) is the critical ligand for transition to the medulla and tolerance induction in T cells.<sup>201,202</sup> CCR7 also cooperates with CXCR4 signals that mediate the migration to the thymic cortex<sup>203</sup> while CCR9 plays a non-redundant role for the transition from the cortex to the subcapsular area.<sup>204</sup> The differential role of these chemokine receptors during the migrational steps of thymocytes is reflected by their dynamic expression pattern during the developmental stages (Figure 2A).<sup>205</sup> Vice versa blocking the migration of thymocytes in turn alters the development of the thymus and its functional organization.<sup>196</sup>

After leaving the thymus T cells commence their recirculation between SLO that is regulated by S1PR1 as discussed above. However, on the second look it becomes clear that multiple GPCRs are involved in this process that act in an opposing fashion. Once T cells enter the LN they stay for about 6-24 hours depending on the cell type—CD4 T cells dwell shorter than CD8 T cells, and Tcm longer than naive T cells.<sup>206,207</sup> As mentioned above, time-dependent resensitization of S1PR1 in SLO promotes the exit to the lymph over time. Interestingly, CCR7 heterozygous lymphocytes have shortened dwell times in the LN, while CCR7 overexpressing or S1PR1 heterozygous T cells stayed significantly longer.<sup>208</sup> This argues for a competitive situation between retentions signals (via CCR7 and

CXCR4) and exit cues (via S1PR1) that determine overall T-cell dwell times in LNs (Figures 3C and 4). Competitive GPCR signals are likely a typical scenario that T cells experience while exiting other tissues as well like the bone marrow or the thymus. But also during an immune response, in particular T follicular helper cells (Tfh) are exposed to competitive GPCR signals like CXCR5- and CCR7-mediated chemotactic cues.<sup>209</sup> While it is highly likely that other T cells are similarly exposed to conflicting GPCR signals during inflammatory conditions, very little is known how such competition regulates lymphocyte trafficking *in vivo*.

Upon antigen-specific activation by DCs, naive T cells undergo dramatic transcriptional changes, proliferate vigorously, and differentiate into a continuum of cellular states ranging from short-lived effector cells on one end to long-lived memory cells at the other end of the spectrum.<sup>210,211</sup> Naive CD4 T cells are further specialized to develop into different classes of T-helper cell subsets like Th1, Th2, Th17, Tfh, and Treg cells as reviewed elsewhere.<sup>212,213</sup> Notably, these different classes of T-helper cells reflect specialized cellular states that are geared toward combating different types of infections (intracellular pathogens, extracellular pathogens, parasites, and fungi), helping to produce neutralizing antibodies or controlling overt immune responses and immunopathology. Naturally, these tasks require the migration to different tissues, microanatomical niches, and specific inflammatory environments. In line with this notion, different T-helper subsets express specific patterns of chemokine receptors that predominantly guide them to the required target site.<sup>214</sup> Over the last decade this has been intensively investigated for Tfh. Together, several studies have revealed a highly coordinated and finely tuned microanatomical positioning of these cells during an emerging antibody response.<sup>209,215</sup> With regard to the developing immune responses that give rise to Th1, Th2, and Th17 cells much less is known in terms of how chemokines regulate the positioning of these cells during their priming and differentiation. Clearly, chemokines play an important role here as well, as exemplified by the function of CXCR3 for Th1 differentiation.<sup>216</sup> As Treg cells must be able to control the various flavors of elicited immune responses and their cellular representatives, Tregs must follow (or precede) different effector cells to their specific inflammatory sites and microanatomical niches.<sup>217,218</sup> In order to do so, they partially adopt the transcriptional programs of their respective T-helper cell counterparts.<sup>219-221</sup> Overall, temporal and spatial control of GPCR signaling in T cells is critical on two levels: (a) it guides their differentiation in SLO and (b) it directs them to and within inflamed tissues where they execute their specific functions (Figure 3E). In the following paragraph we will focus on cytotoxic CD8 T cells, how their chemokine expression pattern changes during activation, in which way it differs among CD8 T cells subsets, and how it impacts on their differentiation.

Once naive CD8 T cells exit the thymus they commence their recirculation pattern through the body predominantly via the GPCRs CXCR4, CCR7, and S1PR1 as discussed above. Additionally and by contrast to naive CD4 T cells, naive CD8 T cells express high levels of CCR9<sup>222,223</sup> and intermediate levels of CXCR6.<sup>224</sup> To date, the exact function of these chemokine receptors on naive CD8 T cells

remains unknown. Upon activation, CD8 T cells change their GPCR repertoire. During their differentiation into effector and memory subsets specific GPCR patterns are established over time (Figure 6). Accordingly, we can group different GPCRs based on their temporal expression pattern. We can further discern four phases during which GPCRs execute specific biological functions. Before activation, the time frame after activation but before T cells commence proliferation, T-cell effector phase, and T-cell memory phase. Naive T cells express GPCRs that are important for their recirculation but additionally may help them to efficiently detect antigen-presenting DC. In particular, CXCR6, Gpr183 (Ebi2), and CCR9 could support this process. Once a CD8 T cell has engaged an antigen-presenting DC it co-arrests and interacts with it for several hours.<sup>225-228</sup> This activation process is accompanied by the expression of additional GPCRs while the expression of other GPCRs may be shutdown. Upon disengagement from the DC, now activated CD8 T cells become receptive to a newly formed inflammatory and chemotactic environment and change their localization within the reactive lymph node. This in turn will influence their interaction partners in particular cDC over the following day before they proliferate and exit the lymph node. Importantly, there is strong evidence that T cells integrate these consecutive interactions with DC following the initial priming step.<sup>172,229,230</sup> Therefore, both the initial priming and the consecutive encounters of CD8 T cells with DC will shape the size and the differentiation of the ensuing CD8 T-cell response. The chemokine receptors that are upregulated during this phase are CCR5 and CXCR3. Similar as in pDC as we will discuss below, these chemokine receptors appear to direct T cells to different localizations of the lymph node. Specifically, several studies have indicated that CCR5 guides CD8 T cells to receive helper signals by licensed DC.<sup>178,231,232</sup> We and others have shown that in particular cDC1 relay these helper signals and are located in deeper paracortical areas.<sup>169,229,233</sup> By contrast CXCR3 appears to guide CD8 T cells to the subcapsular sinus of the lymph node and promotes effector T-cell generation.<sup>170,171,234,235</sup> How activated CD8 T cells navigate within inflamed tissues (including the lymph node) through complex chemokine fields and in which situations CCR5- and CXCR3-mediated signals are rather synergistic than conflicting remains largely unknown.<sup>236,237</sup>

By the time that T cells have fully adopted their effector program and leave the lymph node they express additional inflammatory chemokine receptors like CCR2 and CX3CR1. While CXCR3 and CCR5 are expressed by the majority of CD8 T cells at the peak of an immune response, CX3CR1 shows a bimodal expression pattern with about half of the CD8 T cells expressing this GPCR. Interestingly, the expression pattern of CX3CR1 strictly correlates with cytotoxic effector molecules like Prf1 and GrzmB.<sup>238,239</sup> Importantly, CX3CR1 expression not only correlates with a specific effector function but also delineates a specific migratory behavior. It was recently shown that CX3CR1<sup>hi</sup> effector memory CD8 T cells predominantly scan blood vessels rather than migrating within specific tissues with the exception of the spleen. By contrast, CX3CR1<sup>int</sup> memory CD8 T cells that have been termed Tpm (peripheral memory T cells) appear to migrate through peripheral

tissues and enter lymph nodes via afferent lymphatics rather than HEV.<sup>240</sup> GPCRs that help T cells to extravasate into inflamed tissues often act in a redundant manner while in other cases they work synergistically (Figure 3B). Typically, one chemokine receptor can bind to multiple ligands and vice versa one ligand can be bound by multiple receptors.<sup>241</sup> In vitro there are several examples of GPCR synergy (for review see<sup>242</sup>). For T cells in vivo there are only few examples supporting synergy (rather than redundancy) between chemokines. For example, CCL5 and CXCL10 have been shown to synergize in promoting T-cell extravasation, yet in a somewhat artificial model using intradermal injection of chemokines.<sup>243</sup> Besides synergy between CCR5 and CXCR3, other GPCR pairs are likely to cooperate in T cells as CCR7 and CXCR4, CCR2 and CCR6, or CCR4 and CCR8. Overall, time-dependent changes in GPCR patterns in T cells are tightly connected to their specific function, guide them through their developmental steps, and navigate them to and within inflamed tissues. However, how T cells traffick through complex chemokine fields while utilizing multiple GPCRs that may act in synergy or in antagonism is highly complex and is largely unknown.

## 4 | DENDRITIC CELLS

Conventional DCs (cDC) may be regarded as central inducers of adaptive cellular immune responses. DC express a variety of receptors that allow them to sense infections, cellular damage, and metabolic changes.<sup>244-246</sup> Additionally, as phagocytes, DC are able to take up antigen, digest and process it so that it becomes detectable for T lymphocytes in the context of MHC molecules.<sup>247</sup> Reflective of these biological functions, DC are able to integrate inflammatory and antigenic information and translate them into appropriate output signals which initiate a suitable immune response.<sup>248</sup> With regard to GPCR-mediated navigation a key feature of DCs is their migration from peripheral tissues to draining LNs in the context of their functional maturation.<sup>249</sup> In contrast to T cells which are geared to search large tissue volumes, DC need to migrate and to settle within specific anatomical niches of SLO in order to be efficiently encountered by T cells or to be strategically positioned for optimal access to antigenic material.<sup>250</sup> In mice and humans, DC comprise a rather heterogeneous group of cells with differential localization in tissues, different migratory behavior, and functional specialization. We currently discern four major groups of DC,<sup>251</sup> cDC that are further subdivided in cDC1 and cDC2, plasmacytoid DC (pDC) that are characterized by their ability to produce large amounts of IFN $\alpha$  allowing them to support both innate and adaptive immune responses,<sup>252</sup> and monocyte-derived DC (MoDC) that play a critical role during bacterial infections and coordinate effector responses in tissues.<sup>253</sup> Monocytes can differentiate into DC on-site following their migration into inflamed tissues, yet their exact function regarding the development of adaptive immune responses is still under debate.<sup>254</sup> Overall, in order to fulfill their central function in lymphocyte activation, GPCR mediated navigation is a critical requirement for DC.

In vitro assays have been widely used to study DC migration in order to measure their responsiveness toward specific chemoattractants, to determine downstream signaling events and to identify critical molecules that are involved in DC locomotion.<sup>16,255,256</sup> Most of these assays used human MoDCs or mouse bone marrow-derived DC (BMDC) to study their chemotactic responses. BMDC are a very popular immune cell type to study chemokine sensing and cytoskeletal dynamics due to their pronounced lamellopodia and actin-based protrusions. However, they are also seen critical because BMDC cultures comprise highly heterogeneous myeloid cells that only in part resemble DC.<sup>257</sup> Studies on GPCR-controlled DC migration mainly focus on the chemokine receptor CCR7, which becomes highly up-regulated on DCs upon maturation. Moreover, inflammatory signals promote CCR7 oligomerization and promigratory signaling responses in DCs.<sup>39</sup> CCR7 binds two ligands, the chemokines CCL19 and CCL21, which differ structurally.<sup>258</sup> CCL21 harbors a unique extended C-terminal tail that is negatively charged due to stretches of basic amino acids. This allows CCL21 to bind and become immobilized to GAG with high affinity, with the potential to form haptotactic gradients.<sup>6</sup> In contrast, CCL19 lacks this C-terminus and is hence considered to diffuse and form soluble chemotactic gradients. In comparison, CCL21 can form steeper gradients than CCL19.<sup>259</sup> Transferring the CCL21 tail to CCL19 increases GAG binding of CCL19,<sup>260</sup> whereas CCL21 becomes more soluble upon removal of its C-terminus.<sup>261</sup> Interestingly, DC themselves can proteolytically cleave CCL21 to remove its C-tail, turning it into a more diffusible chemotactic molecule.<sup>46,261</sup> Besides these differences in gradient formation, it is now also acknowledged that CCL19 and CCL21 bias CCR7 downstream signaling, desensitization, and receptor dynamics in distinct ways.<sup>262-265</sup> Thus, these aspects of CCR7 functionality have received much attention and spawned the development of various in vitro assays, now allowing the analysis of DC migration in various experimental settings including 3D gels with soluble gradients of altered shapes,<sup>256,266-268</sup> substrate-bound gradients,<sup>269</sup> or combinations thereof.<sup>270</sup> DC navigation in the presence of multiple chemotactic cues mostly focused on competing fields of CCL19 and CCL21.<sup>46,52,270,271</sup> From one of such studies emerged the concept that local soluble CCL19 gradients can direct DC haptokinesis on immobilized CCL21.<sup>46</sup> In addition to CCR7, other GPCRs including CXCR4<sup>52,272,273</sup> and S1P receptors<sup>274</sup> are also functionally relevant for DC migration. When chemoattractive potencies were matched, DCs were found to home to central regions in opposing gradients of the CXCR4 ligand CXCL12 and CCR7 ligands.<sup>52</sup> In the following, we will discuss what we have learned from in vivo studies regarding GPCR-guided DC navigation.

#### 4.1 | Interstitial dynamics: from sessile networks to highly directed pathfinders

In contrast to lymphocytes, chemokinesis appears to play a minor role for the migration of cDC in the steady state (Figure 1A). cDC rather form a sessile network<sup>275</sup> in the lymph node, while newly arriving DC immigrants from the blood or the lymphatics are rapidly

directed to their respective niche by chemo- and haptotactic signals (Figure 1B). However, there are some notable exceptions like CCR6-mediated chemokinesis that leads to the accumulation of cDC around small airways in the lung in the context of inflammation.<sup>276</sup> With regards to their steady-state migration but also their morphology, pDC represent a somewhat intermediate cell type between lymphocytes and cDC. In LNs, pDC migrate in a chemokinetic manner similar to lymphocytes albeit at a significantly reduced speed (5 μm/min) and with less spatial displacement over time.<sup>172</sup> The molecules that regulate LN entry and chemokinetic pDC migration in vivo are CCR7 and CXCR4.<sup>277,278</sup>

The first direct evidence for haptotaxis in guiding leukocyte migration in situ was provided in the context of cDC intravasation into lymphatic vessels (Figure 1B).<sup>6,279</sup> Sixt and coworkers directly visualized CCL21 in ear skin whole mounts and showed that it forms steep gradients around lymphatic vessels. Cells with a sufficient size (like cDC) are able to spatially sense such gradients and migrate directionally toward lymphatic vessels. Importantly, CCL21 is physically attached to the ECM via binding to heparan sulfates and removal via heparitinase altered CCL21 patterning and diminished cDC migration. As almost all chemokines have GAG-binding properties, haptotaxis might be a more generally utilized principle for immune cell migration and in particular for cDC. For example, once DC intravasated into the lymphatic vessel, they follow a lymph-flow induced haptotactic CCL21 gradient until they detach and get passively carried to the dLN.<sup>280</sup> When cDC arrive in the dLN they likely follow CCR7-mediated haptotactic cues in order to enter the LN parenchyma.<sup>281</sup> In line with this notion expression of the atypical chemokine receptor ACKR4 within the lymphatic endothelium has been shown to be critical to shape a CCL21 gradient across the sinus floor.<sup>7</sup> In the absence of ACKR4 migratory DC get trapped within the SCS and fail to enter the LN parenchyma. Haptotactic cues are probably not only guiding cDC to the LN but also help them to find their specific niche within SLO. However, this intranodal trafficking of cDC may also be a combination of haptokinetic and chemotactic signals as we will discuss in more detail below.

#### 4.2 | Orientation in the tissue: Is it all about CCR7 functionality?

In general, cDC immigrate to LNs via two separate entry points. (a) DC precursors that are released from the bone marrow enter the LN via high endothelial venules (HEV) in a CCR7-dependent manner. (b) Tissue residing cDC, in the context of their maturation, upregulate CCR7 and migrate to lymphatics and enter the LN via the SCS and the interfollicular area of the LN. In this section we will focus on DC migrating from tissues to LNs as a prime example of temporal changes in single GPCR expression that regulate cellular migration (Figure 2A).<sup>282</sup>

In the steady state DC undergo a complex maturation program that largely overlaps with pathogen-induced maturation.<sup>283</sup> This homeostatic maturation of DC is operative in the absence of microbiota or IFN I signaling, but depends on NF-κB activation.<sup>284</sup>

A central element of the maturation process is the upregulation of CCR7.<sup>285,286</sup> CCR7 is absolutely required for the migration of cDC to draining LN in the steady state and during inflammation.<sup>287</sup> Notably, dermal DC that enter the LN at the interfollicular zone, settle in the outer-paracortical region that is rich for HEV, thereby enhancing the likelihood to engage with newly arriving T cells that enter the LN from the blood stream via HEV.<sup>288</sup> By contrast dermal Langerhans cells (LC) migrate further into the LN and settle within the deep paracortical region.<sup>289</sup>

Besides migration to SLO, CCR7 upregulation appears to further regulate migration within SLO. In the context of systemic infections, cDC1 translocate from the red pulp to the white pulp of the spleen while cDC2 migrate from the bridging channels toward the white pulp.<sup>49,166</sup> Within LNs, cDC1 translocate from the interfollicular area to the paracortex upon viral infections, which again is likely mediated via CCR7.<sup>172</sup> By contrast, LN-resident cDC2 translocate to the medullary area and then further to the interfollicular zones to activate CD4 lymphocytes in context of influenza infections.<sup>290</sup> While some GPCR like GPR183, CXCR5, and XCR1 help to lodge DC into specific niches of the LN or direct them to certain areas upon inflammation, DC migration is overall dominated CCR7-mediated signals and consequently by changes in surface expression of this receptor. As during lymphocyte recirculation, receptor desensitization adds an additional layer fine-tuning the regulation of DC migration (Figure 2D). Homologous desensitization for CCR7 that is highly relevant for DC trafficking has been shown to be ligand dependent. In the case of CCL19, both GRK3 and GRK6 are activated leading to CCR7 internalization and recycling and overall to receptor desensitization.<sup>263,291</sup> By contrast CCL21 only activates GRK6 and does not induce receptor internalization arguing for an alternative way of CCR7 desensitization.<sup>265</sup> In vitro, GRK6-deficient DC failed to migrate toward haptotactic cues in particular at low CCL21 concentrations. In vivo, the situation is less clear. While fewer GRK6-deficient DC are able to immigrate into ear skin explants than WT DC, these KO DC intravasated more efficiently into the lymphatic vessels.<sup>269</sup>

Upon GRK-mediated phosphorylation of the GPCR, arrestins get recruited initiating the actual desensitization process.<sup>292</sup> In particular,  $\beta$ -arrestin 2 has been shown to regulate the sensitivity toward CCR7 and CXCR4 mediated cues. Functionally,  $\beta$ -arrestin 2 deficiency in DC resulted in enhanced steady-state migration to SLO and aggravated disease status in various autoimmune models.<sup>293</sup> On a molecular level,  $\beta$ -arrestins, like GRKs seem to act beyond receptor desensitization leading to more complex phenotypes that cannot be explained by alterations in migratory behavior alone.

In summary, modification of GPCR surface expression and sensitivity regulation are integral elements of DC migration and function, allowing them to mount and fine-tune adaptive immunity toward infections via compartmentalization of leukocyte interactions.<sup>294</sup>

As discussed above, the migration of DC from tissues to the dLN is a process that is dominated by CCR7-mediated guidance cues. But not only CCR7 is dominant as a receptor also regarding its ligands CCL19 and CCL21 there is a clear hierarchy in vivo. In particular, it was shown that CCL19-deficient animals show normal DC migration

to and localization within LNs.<sup>295</sup> However, regarding the migration to LNs there are two interesting situations that require additional synergistic and sequential GPCR signals for efficient trafficking to the LN (Figure 3B,E). One example are LC that first need to detach<sup>296</sup> from the epidermis and as an initial migratory step, need to cross the basal membrane and migrate to the dermis. This primary migratory event appears to be independent of CCR7 but rather utilizes various context-dependent GPCRs.<sup>297-299</sup> While LC migrate to the dermis they upregulate CCR7 and enter lymphatic vessel similar to cDC in a secondary migratory step. Consequently, LC require significantly more time to emigrate from the skin than dermal cDC. Interestingly, it has been recently demonstrated that a subset of dermal DC also shows a two-step migration pattern.<sup>300,301</sup> In particular, CD301b expressing dermal DC require CCR8 in the context of Th2 priming conditions in order to cross the subcapsular sinus of the LN. In this situation, CCR8 does not promote chemotaxis itself but rather potentiates the effect of CCR7-mediated migration. A similar requirement for CCR8 has been observed for the migration of moDC from the skin to draining LNs.<sup>302</sup> However, why the migration of CD301b DC and moDC, in contrast to other dermal DC subsets, is regulated via this second migration step and why CCR7-mediated signals are sufficient to drive emigration of these DC from the skin, yet insufficient to promote the full immigration to the LN requires further investigations.

While DC migration to the dLN is overall dominated by CCR7,<sup>303</sup> in tissues multiple GPCRs can get activated in order to lodge DC into their optimal niche. This lodging within tissues could be based on chemotactic signals on top of CCR7-mediated haptokinetic cues (Figure 3A,B,E). Some prominent examples are (a) GPR183-mediated migration, which navigates cDC to the bridging channel in the spleen or the T/B border of the LN,<sup>167,168</sup> and (b) the CXCR5-dependent cDC migration to the interfollicular and subcapsular sinus area of the LN in the context of *H. pylori* infections,<sup>304</sup> as well as (c) CCR6-mediated guidance of cDC to the subepithelial dome of Peyer's patches<sup>305</sup> and (d) cDC migration from the subepithelial dome to the T/B border upon activation and infection<sup>306</sup> or (e) intranodal migration of cDC1 toward activated CD8 T cells following a gradient of XCL1<sup>172</sup> and skin DC clustering around macrophages based on CXCL2-mediated recruitment.<sup>307</sup> In order to resolve the exact mode(s) of migration in these scenarios, it will be required to determine whether the chemoattractants at play are soluble or immobilized and how DC interpret combinations of chemokines in these situations.

In contrast to cDC that predominantly express homeostatic GPCRs, pDC are additionally equipped with inflammatory GPCRs like CCR2, CCR5, and CXCR3. This is reminiscent to NK cells, ILC1, Th1 cells, and effector CD8 T cells, cell types that cooperate to eliminate intracellular pathogens. In the steady state pDC that reside in SLO are primarily localized in the paracortex, yet appear to be enriched at the interfollicular area of the LN similar to memory CD8 T cells.<sup>170,172</sup> Interestingly, when we analyzed the migratory behavior of pDC during viral infections, we found that pDC accumulate at two different anatomical sites of the LN already a few hours after infection. One group of pDC migrates to the subcapsular sinus

area where they interact with virally infected sinus-lining macrophages.<sup>172,308</sup> By contrast, a second group of pDC migrates toward recently activated CD8 T cells that interact with antigen-presenting cDC. Mechanistically, these sites emit different chemokine cues exposing the responding cells to competitive signals (Figures 3C and 4). The SCS area releases IFN-induced CXCR3 ligands (CXCL9, CXCL10, and CXCL11) upon viral infection. While activated CD8 T cells produce CCL3 and CCL4 that recruit pDC via CCR5. Interestingly, this migratory ambivalence also reflects two different biological functions of pDC. CXCR3-guided pDC release IFN $\alpha$  at the site of infection in order to limit viral replication and dissemination. CCR5-guided pDC release IFN $\alpha$  at the site of CD8 T-cell priming, which promotes the maturation and cross-presentation capacity of cDC1. In other words, one group of pDC serves an innate function, while another group supports adaptive immunity—notably via the same effector function, yet within different microanatomical compartments. This dichotomous navigation of groups of cells between conflicting signals was not only seen for pDC but also for other cell types that are equipped with the same GPCRs like NK cells (unpublished observation W. Kastentmüller). This poses the question how the immune system generally operates in order to ensure rapid and robust arrival of one cell type at two different sites within a tissue? A strictly hierarchical system is not in line with the observed phenotype because it would predict that all cells end up at one site (Figure 4A). So, some decision process is required assuming that both GPCRs are expressed at a functional level, otherwise cells could get paralyzed or disorientated between opposing gradients (Figure 4B). While CCR5 and CXCR3 drive pDC to different areas of the inflamed LN, these same GPCRs rather show synergistic functions on T cells.<sup>237</sup> On the other hand, it is quite likely that cell type-specific differences exist and that some GPCR's synergize in one cell type, yet may have cross-inhibiting functions in others. In fact, even on a single cell level, heterogeneity between cells may contribute to the “decision-making” process in conflicting chemokine fields (Figure 4C). The assumption that both GPCRs (CCR5 and CXCR3) are expressed homogeneously on pDC is rather unlikely and instead a range of expression patterns across a population may underlie the single cell behavior seen in vivo. In other words, pDC may not integrate opposing signals while they migrate through tissues but are rather geared to preferentially follow certain cues a priori. This preference may be further hard wired by downstream or inhibitory molecules of the respective GPCR signaling pathway.

While some chemokine gradients may drive DC to opposing sites, others may synergize to optimize the recruitment process (Figure 3A,B). However, it is often difficult to discern between redundancy vs synergy of GPCRs as many in vivo studies rely on end-point analyses of gene-deficient animals. However, there are a couple of examples that likely reflect synergistic effects of GPCR signaling in guiding DC migration in vivo. It has been shown that the abundance of cDC1 and expression of CCR7 within tumors is a prognostic marker of clinical outcomes.<sup>309,310</sup> Recent evidence supports a critical role for intratumoral NK cells in recruiting cDC1 within the tumor which is essential for effective immune responses against

cancer.<sup>311</sup> This recruitment appears to be based on synergistic effects of NK cell-derived CCL5 and XCL1. Another example is the role of CCL17 for DC emigration from the skin in the context of Th2 responses.<sup>299</sup> In vitro, DC-derived CCL17 synergizes with CCR7-mediated signals to optimize directionality and speed of DC migration. Mechanistically, this synergistic effect is not based on altered surface expression of CCR7 in the presence or absence of CCL17 but rather on downstream signaling events involving enhanced Ca<sup>2+</sup> flux. In vivo this results in a significant reduction in DC migration to dLN in the absence DC-derived CCL17. Finally, pDC trafficking into the white pulp of the spleen was shown to be based on a cooperative action between CCR7 and CXCR4.<sup>277</sup>

Overall cDC migration seems to be dominated by CCR7-mediated navigation reflecting the functional maturation of DC and trafficking from tissues to dLNs.<sup>249</sup> However, at a closer look we find that DC fine-tune their migratory behavior using multiple GPCR in order to populate specific microanatomical niches that are critical to mount adaptive immunity or to orchestrate effector responses in tissues.<sup>172,307,311</sup>

## 5 | CONCLUSION

In vivo cellular migration is a highly complex process and we observe different migratory patterns dependent on the chemoattractant, the engaged GPCR, intracellular regulatory proteins, the leukocyte subset, and the nature of the tissue. Some of the observed migratory patterns can be well explained by single GPCRs that may dominate under specific conditions. In reality, however, there are typically several GPCRs engaged simultaneously or sequentially and the responding cells need to integrate these signals. Some of these signals may be synergistic, while others are conflicting. Adding to the intricacy is the fact that some chemokines are agonistic to one receptor, yet maybe antagonistic to another one.<sup>312</sup> Additionally, MMP-mediated degradation of agonistic chemokines can result in products that are antagonistic to the same chemokine receptor.<sup>313</sup> This raises important questions on how cells navigate through complex GPCR fields without getting trapped and paralyzed between opposing signals or get distracted and follow the wrong path. What is the role of single cell heterogeneity to promote flexibility of the system without sacrificing its robustness? Dynamic changes in GPCR expression patterns upon cellular activation and concomitant alterations of chemotactic cues and biophysical features of inflamed tissues add yet another layer of complexity that cells need to resolve in order to be guided to the right location in the shortest amount of time.

Biologically, navigation of leukocytes through inflamed tissues to infected sites and local execution of their specific effector function and importantly also termination of such a response reflects the balance between pathogen clearance and tissue damage. It is this very balance that determines the healthy vs the diseased state of an individual. Revealing how leukocytes exactly regulate their trafficking through complex chemoattractant fields will be extremely challenging. On one hand we lack information regarding



the precise nature of the chemokine fields at work. How many cells produce a given chemokine during a specific time frame? How long does the chemokine gradient remains active? How do secondary changes (degradation, modification, or consumption) impact on the chemokine gradient? Novel optogenetic tools may provide us with the capability to sharply control chemokine release *in situ*.<sup>314</sup> With such approaches we will be able to directly control and experimentally influence leukocyte migration *in vivo* instead of being mere observers. On the other hand, we still lack detailed data on how specific GPCRs modulate the migratory behavior of different cell types in different tissues. In many studies the migratory behavior of leukocytes is not directly visualized. Instead, conclusions were drawn based on “end-point” differences using animals that are deficient for a specific GPCR. Optimally, loss- and gain-of-function conditions should be visualized directly side-by-side *in vivo*. GPCRs coupled to fluorescent proteins will further allow us to visualize GPCR internalization and desensitization events. With new tools being developed, continuous technical advancements in intravital microscopy and artificial intelligence supporting the analysis and modeling of imaging data we are confident that many obstacles will be overcome.

## ACKNOWLEDGEMENTS

This effort was supported by the Max Planck Society (T.L.), the German Research Foundation DFG (T.L. and W.K.), and the European Research Council (StG 715890 to T.L. and CoG 819329 to W.K.). We thank Georg Gasteiger and members of the KamuLab for critical reading.

## ORCID

Wolfgang Kastentmüller  <https://orcid.org/0000-0002-3835-1485>

## REFERENCES

- Legler DF, Thelen M. New insights in chemokine signaling. *F1000Res*. 2018;7:95.
- Zabel BA, Rott A, Butcher EC. Leukocyte chemoattractant receptors in human disease pathogenesis. *Annu Rev Pathol*. 2015;10:51-81.
- Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokines grammar for immune cells. *Annu Rev Immunol*. 2004;22:891-928.
- Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol*. 2014;32:659-702.
- Cyster JG. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu Rev Immunol*. 2005;23:127-159.
- Weber M, Hauschild R, Schwarz J, et al. Interstitial dendritic cell guidance by haptotactic chemokine gradients. *Science*. 2013;339:328-332.
- Ulvmar MH, Werth K, Braun A, et al. The atypical chemokine receptor CCRL1 shapes functional CCL21 gradients in lymph nodes. *Nat Immunol*. 2014;15:623-630.
- Germain RN, Robey EA, Cahalan MD. A decade of imaging cellular motility and interaction dynamics in the immune system. *Science*. 2012;336:1676-1681.
- Cahalan MD, Parker I. Choreography of cell motility and interaction dynamics imaged by two-photon microscopy in lymphoid organs. *Annu Rev Immunol*. 2008;26:585-626.
- Deng Q, Huttenlocher A. Leukocyte migration from a fish eye's view. *J Cell Sci*. 2012;125:3949-3956.
- Evans IR, Wood W. Drosophila blood cell chemotaxis. *Curr Opin Cell Biol*. 2014;30:1-8.
- Sanchez-Madrid F, del Pozo MA. Leukocyte polarization in cell migration and immune interactions. *EMBO J*. 1999;18:501-511.
- Cotton M, Claing A. G protein-coupled receptors stimulation and the control of cell migration. *Cell Signal*. 2009;21:1045-1053.
- Gambardella L, Vermeren S. Molecular players in neutrophil chemotaxis—focus on PI3K and small GTPases. *J Leukoc Biol*. 2013;94:603-612.
- Lammermann T, Sixt M. Mechanical modes of ‘amoeboid’ cell migration. *Curr Opin Cell Biol*. 2009;21:636-644.
- Lammermann T, Germain RN. The multiple faces of leukocyte interstitial migration. *Semin Immunopathol*. 2014;36:227-251.
- Zigmond SH, Levitsky HI, Kreel BJ. Cell polarity: an examination of its behavioral expression and its consequences for polymorphonuclear leukocyte chemotaxis. *J Cell Biol*. 1981;89:585-592.
- Worbs T, Mempel TR, Bolter J, von Andrian UH, Forster R. CCR7 ligands stimulate the intranodal motility of T lymphocytes *in vivo*. *J Exp Med*. 2007;204:489-495.
- Vianello F, Olszak IT, Poznansky MC. Fugetaxis: active movement of leukocytes away from a chemokinetic agent. *J Mol Med (Berl)*. 2005;83:752-763.
- Huttenlocher A, Poznansky MC. Reverse leukocyte migration can be attractive or repulsive. *Trends Cell Biol*. 2008;18:298-306.
- Cyster JG. Chemorepulsion and thymocyte emigration. *J Clin Invest*. 2002;109:1011-1012.
- Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. *FEBS J*. 2018;285:2944-2971.
- Rot A. Chemokine patterning by glycosaminoglycans and interceptors. *Front Biosci (Landmark Ed)*. 2010;15:645-660.
- Sarris M, Sixt M. Navigating in tissue mazes: chemoattractant interpretation in complex environments. *Curr Opin Cell Biol*. 2015;36:93-102.
- Bennett LD, Fox JM, Signoret N. Mechanisms regulating chemokine receptor activity. *Immunology*. 2011;134:246-256.
- Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte trafficking. *Am J Physiol Regul Integr Comp Physiol*. 2002;283:R7-R28.
- Kehrl JH. The impact of RGS and other G-protein regulatory proteins on Gα<sub>βγ</sub>-mediated signaling in immunity. *Biochem Pharmacol*. 2016;114:40-52.
- Smith JS, Lefkowitz RJ, Rajagopal S. Biased signalling: from simple switches to allosteric microprocessors. *Nat Rev Drug Discov*. 2018;17:243-260.
- Arai H, Tsou CL, Charo IF. Chemotaxis in a lymphocyte cell line transfected with C-C chemokine receptor 2B: evidence that directed migration is mediated by betagamma dimers released by activation of Gα<sub>βγ</sub>-coupled receptors. *Proc Natl Acad Sci USA*. 1997;94:14495-14499.
- Neptune ER, Iiri T, Bourne HR. Gα<sub>βγ</sub> is not required for chemotaxis mediated by Gi-coupled receptors. *J Biol Chem*. 1999;274:2824-2828.
- Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. *Annu Rev Biochem*. 1998;67:653-692.
- Steury MD, McCabe LR, Parameswaran N. G Protein-coupled receptor kinases in the inflammatory response and signaling. *Adv Immunol*. 2017;136:227-277.
- Liggett SB. Phosphorylation barcoding as a mechanism of directing GPCR signaling. *Sci Signal*. 2011;4:pe36.
- Freedman NJ, Lefkowitz RJ. Desensitization of G protein-coupled receptors. *Recent Prog Horm Res*. 1996;51:319-351; discussion 52-3.



35. Borroni EM, Mantovani A, Locati M, Bonecchi R. Chemokine receptors intracellular trafficking. *Pharmacol Ther.* 2010;127:1-8.
36. Shukla AK, Xiao K, Lefkowitz RJ. Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling. *Trends Biochem Sci.* 2011;36:457-469.
37. Zweemer AJ, Toraskar J, Heitman LH, Ap IJ. Bias in chemokine receptor signalling. *Trends Immunol.* 2014;35:243-252.
38. Borregaard N, Sorensen OE, Theilgaard-Monch K. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol.* 2007;28:340-345.
39. Hauser MA, Schaeuble K, Kindinger I, et al. Inflammation-induced CCR7 oligomers form scaffolds to integrate distinct signaling pathways for efficient cell migration. *Immunity.* 2016;44:59-72.
40. Sica A, Saccani A, Borsatti A, et al. Bacterial lipopolysaccharide rapidly inhibits expression of C-C chemokine receptors in human monocytes. *J Exp Med.* 1997;185:969-974.
41. Doroshenko T, Chaly Y, Savitskiy V, et al. Phagocytosing neutrophils down-regulate the expression of chemokine receptors CXCR1 and CXCR2. *Blood.* 2002;100:2668-2671.
42. Heit B, Tavener S, Raharjo E, Kubes P. An intracellular signaling hierarchy determines direction of migration in opposing chemotactic gradients. *J Cell Biol.* 2002;159:91-102.
43. Rose JJ, Foley JF, Murphy PM, Venkatesan S. On the mechanism and significance of ligand-induced internalization of human neutrophil chemokine receptors CXCR1 and CXCR2. *J Biol Chem.* 2004;279:24372-24386.
44. Volpe S, Cameroni E, Moepps B, Thelen S, Apuzzo T, Thelen M. CCR2 acts as scavenger for CCL2 during monocyte chemotaxis. *PLoS ONE.* 2012;7:e37208.
45. Foxman EF, Campbell JJ, Butcher EC. Multistep navigation and the combinatorial control of leukocyte chemotaxis. *J Cell Biol.* 1997;139:1349-1360.
46. Schumann K, Lammernann T, Bruckner M, et al. Immobilized chemokine fields and soluble chemokine gradients cooperatively shape migration patterns of dendritic cells. *Immunity.* 2010;32:703-713.
47. Byrne MB, Kimura Y, Kapoor A, et al. Oscillatory behavior of neutrophils under opposing chemoattractant gradients supports a winner-take-all mechanism. *PLoS ONE.* 2014;9:e85726.
48. Nandagopal S, Wu D, Lin F. Combinatorial guidance by CCR7 ligands for T lymphocytes migration in co-existing chemokine fields. *PLoS ONE.* 2011;6:e18183.
49. Boneschansker L, Jorgensen J, Ellett F, Briscoe DM, Irimia D. Convergent and divergent migratory patterns of human neutrophils inside microfluidic mazes. *Sci Rep.* 2018;8:1887.
50. Afonso PV, Janka-Junttila M, Lee YJ, et al. LTB4 is a signal-relay molecule during neutrophil chemotaxis. *Dev Cell.* 2012;22:1079-1091.
51. Foxman EF, Kunkel EJ, Butcher EC. Integrating conflicting chemotactic signals. The role of memory in leukocyte navigation. *J Cell Biol.* 1999;147:577-588.
52. Ricart BG, John B, Lee D, Hunter CA, Hammer DA. Dendritic cells distinguish individual chemokine signals through CCR7 and CXCR4. *J Immunol.* 2011;186:53-61.
53. Ali H, Richardson RM, Haribabu B, Snyderman R. Chemoattractant receptor cross-desensitization. *J Biol Chem.* 1999;274:6027-6030.
54. Heit B, Robbins SM, Downey CM, et al. PTEN functions to 'prioritize' chemotactic cues and prevent 'distraction' in migrating neutrophils. *Nat Immunol.* 2008;9:743-752.
55. Bachelerie F, Graham GJ, Locati M, et al. New nomenclature for atypical chemokine receptors. *Nat Immunol.* 2014;15:207-208.
56. Rot A, McKimmie C, Burt CL, et al. Cell-autonomous regulation of neutrophil migration by the D6 chemokine decoy receptor. *J Immunol.* 2013;190:6450-6456.
57. Humpert ML, Pinto D, Jarrossay D, Thelen M. CXCR7 influences the migration of B cells during maturation. *Eur J Immunol.* 2014;44:694-705.
58. Bonecchi R, Locati M, Galliera E, et al. Differential recognition and scavenging of native and truncated macrophage-derived chemokine (macrophage-derived chemokine/CC chemokine ligand 22) by the D6 decoy receptor. *J Immunol.* 2004;172:4972-4976.
59. Ley K, Hoffman HM, Kubes P, et al. Neutrophils: new insights and open questions. *Sci Immunol.* 2018;3:pii: eaat4579.
60. Nicolas-Avila JA, Adrover JM, Hidalgo A. Neutrophils in homeostasis, immunity, and cancer. *Immunity.* 2017;46:15-28.
61. Adrover JM, Nicolas-Avila JA, Hidalgo A. Aging: a temporal dimension for neutrophils. *Trends Immunol.* 2016;37:334-345.
62. Casanova-Acebes M, Nicolas-Avila JA, Li JL, et al. Neutrophils instruct homeostatic and pathological states in naive tissues. *J Exp Med.* 2018;215:2778-2795.
63. Strydom N, Rankin SM. Regulation of circulating neutrophil numbers under homeostasis and in disease. *J Innate Immun.* 2013;5:304-314.
64. Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat Rev Mol Cell Biol.* 2010;11:366-378.
65. Lammernann T. In the eye of the neutrophil swarm-navigation signals that bring neutrophils together in inflamed and infected tissues. *J Leukoc Biol.* 2016;100:55-63.
66. Kienle K, Lammernann T. Neutrophil swarming: an essential process of the neutrophil tissue response. *Immunol Rev.* 2016;273:76-93.
67. Sadik CD, Kim ND, Luster AD. Neutrophils cascading their way to inflammation. *Trends Immunol.* 2011;32:452-460.
68. McDonald B, Kubes P. Cellular and molecular choreography of neutrophil recruitment to sites of sterile inflammation. *J Mol Med (Berl).* 2011;89:1079-1088.
69. Futosi K, Fodor S, Mocsai A. Neutrophil cell surface receptors and their intracellular signal transduction pathways. *Int Immunopharmacol.* 2013;17:638-650.
70. Tomhave ED, Richardson RM, Didsbury JR, Menard L, Snyderman R, Ali H. Cross-desensitization of receptors for peptide chemoattractants. Characterization of a new form of leukocyte regulation. *J Immunol.* 1994;153:3267-3275.
71. Kitayama J, Carr MW, Roth SJ, Buccola J, Springer TA. Contrasting responses to multiple chemotactic stimuli in transendothelial migration: heterogeneous desensitization in neutrophils and augmentation of migration in eosinophils. *J Immunol.* 1997;158:2340-2349.
72. Campbell JJ, Foxman EF, Butcher EC. Chemoattractant receptor cross talk as a regulatory mechanism in leukocyte adhesion and migration. *Eur J Immunol.* 1997;27:2571-2578.
73. Lin F, Nguyen CM, Wang SJ, Saadi W, Gross SP, Jeon NL. Neutrophil migration in opposing chemoattractant gradients using microfluidic chemotaxis devices. *Ann Biomed Eng.* 2005;33:475-482.
74. Sadik CD, Kim ND, Iwakura Y, Luster AD. Neutrophils orchestrate their own recruitment in murine arthritis through C5aR and FcγR signaling. *Proc Natl Acad Sci USA.* 2012;109:E3177-E3185.
75. Reategui E, Jalali F, Khankhel AH, et al. Microscale arrays for the profiling of start and stop signals coordinating human-neutrophil swarming. *Nat Biomed Eng.* 2017;1:0094.
76. Kim D, Haynes CL. Neutrophil chemotaxis within a competing gradient of chemoattractants. *Anal Chem.* 2012;84:6070-6078.
77. Moussavi-Harami SF, Pezzi HM, Huttenlocher A, Beebe DJ. Simple microfluidic device for studying chemotaxis in response to dual gradients. *Biomed Microdev.* 2015;17:9955.
78. Cho H, Hamza B, Wong EA, Irimia D. On-demand, competing gradient arrays for neutrophil chemotaxis. *Lab Chip.* 2014;14:972-978.
79. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. *J Immunol.* 2007;178:3912-3917.

80. Rajarathnam K, Schnoor M, Richardson RM, Rajagopal S. How do chemokines navigate neutrophils to the target site: Dissecting the structural mechanisms and signaling pathways. *Cell Signal*. 2019;54:69-80.
81. Zarbock A, Deem TL, Burcin TL, Ley K. Galphai2 is required for chemokine-induced neutrophil arrest. *Blood*. 2007;110:3773-3779.
82. Kavelaars A, Vroon A, Raatgever RP, et al. Increased acute inflammation, leukotriene B4-induced chemotaxis, and signaling in mice deficient for G protein-coupled receptor kinase 6. *J Immunol*. 2003;171:6128-6134.
83. Fan J, Malik AB. Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. *Nat Med*. 2003;9:315-321.
84. Su Y, Raghuvanshi SK, Yu Y, Nanney LB, Richardson RM, Richmond A. Altered CXCR2 signaling in beta-arrestin-2-deficient mouse models. *J Immunol*. 2005;175:5396-5402.
85. Raghuvanshi SK, Su Y, Singh V, Haynes K, Richmond A, Richardson RM. The chemokine receptors CXCR1 and CXCR2 couple to distinct G protein-coupled receptor kinases to mediate and regulate leukocyte functions. *J Immunol*. 2012;189:2824-2832.
86. Cho H, Kamenyeva O, Yung S, et al. The loss of RGS protein-Galphai2 interactions results in markedly impaired mouse neutrophil trafficking to inflammatory sites. *Mol Cell Biol*. 2012;32:4561-4571.
87. Vroon A, Heijnen CJ, Raatgever R, et al. GRK6 deficiency is associated with enhanced CXCR4-mediated neutrophil chemotaxis in vitro and impaired responsiveness to G-CSF in vivo. *J Leukoc Biol*. 2004;75:698-704.
88. Chan EC, Ren C, Xie Z, et al. Regulator of G protein signaling 5 restricts neutrophil chemotaxis and trafficking. *J Biol Chem*. 2018;293:12690-12702.
89. Tharp WG, Yadav R, Irimia D, et al. Neutrophil chemorepulsion in defined interleukin-8 gradients in vitro and in vivo. *J Leukoc Biol*. 2006;79:539-554.
90. De Filippo K, Rankin SM. CXCR4, the master regulator of neutrophil trafficking in homeostasis and disease. *Eur J Clin Invest*. 2018;48(Suppl 2):e12949.
91. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity*. 2006;25:977-988.
92. Mendez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. *Nature*. 2008;452:442-447.
93. Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity*. 2003;19:583-593.
94. Petty JM, Lenox CC, Weiss DJ, Poynter ME, Suratt BT. Crosstalk between CXCR4/stromal derived factor-1 and VLA-4/VCAM-1 pathways regulates neutrophil retention in the bone marrow. *J Immunol*. 2009;182:604-612.
95. Semerad CL, Liu F, Gregory AD, Stumpf K, Link DC. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. *Immunity*. 2002;17:413-423.
96. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest*. 2010;120:2423-2431.
97. Wengner AM, Pitchford SC, Furze RC, Rankin SM. The coordinated action of G-CSF and ELR + CXC chemokines in neutrophil mobilization during acute inflammation. *Blood*. 2008;111:42-49.
98. Kohler A, De Filippo K, Hasenberg M, et al. G-CSF-mediated thrombopoietin release triggers neutrophil motility and mobilization from bone marrow via induction of Cxcr2 ligands. *Blood*. 2011;117:4349-4357.
99. Eash KJ, Means JM, White DW, Link DC. CXCR4 is a key regulator of neutrophil release from the bone marrow under basal and stress granulopoiesis conditions. *Blood*. 2009;113:4711-4719.
100. Tzeng YS, Li H, Kang YL, Chen WC, Cheng WC, Lai DM. Loss of Cxcl12/Sdf-1 in adult mice decreases the quiescent state of hematopoietic stem/progenitor cells and alters the pattern of hematopoietic regeneration after myelosuppression. *Blood*. 2011;117:429-439.
101. Liu Q, Li Z, Gao JL, et al. CXCR4 antagonist AMD3100 redistributes leukocytes from primary immune organs to secondary immune organs, lung, and blood in mice. *Eur J Immunol*. 2015;45:1855-1867.
102. Dar A, Schajnovitz A, Lapid K, et al. Rapid mobilization of hematopoietic progenitors by AMD3100 and catecholamines is mediated by CXCR4-dependent SDF-1 release from bone marrow stromal cells. *Leukemia*. 2011;25:1286-1296.
103. Dar A, Goichberg P, Shinder V, et al. Chemokine receptor CXCR4-dependent internalization and resecretion of functional chemokine SDF-1 by bone marrow endothelial and stromal cells. *Nat Immunol*. 2005;6:1038-1046.
104. Devi S, Wang Y, Chew WK, et al. Neutrophil mobilization via plerixafor-mediated CXCR4 inhibition arises from lung demargination and blockade of neutrophil homing to the bone marrow. *J Exp Med*. 2013;210:2321-2336.
105. Hernandez PA, Gorlin RJ, Lukens JN, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet*. 2003;34:70-74.
106. Murphy PM, Heusinkveld L. Multisystem multitasking by CXCL12 and its receptors CXCR4 and ACKR3. *Cytokine*. 2018;109:2-10.
107. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. *Immunity*. 2014;41:694-707.
108. Sanz MJ, Kubes P. Neutrophil-active chemokines in in vivo imaging of neutrophil trafficking. *Eur J Immunol*. 2012;42:278-283.
109. Smith ML, Olson TS, Ley K. CXCR2- and E-selectin-induced neutrophil arrest during inflammation in vivo. *J Exp Med*. 2004;200:935-939.
110. Oynebraten I, Bakke O, Brandtzaeg P, Johansen FE, Haraldsen G. Rapid chemokine secretion from endothelial cells originates from 2 distinct compartments. *Blood*. 2004;104:314-320.
111. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nat Rev Immunol*. 2007;7:803-815.
112. Proebstl D, Voisin MB, Woodfin A, et al. Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. *J Exp Med*. 2012;209:1219-1234.
113. Abtin A, Jain R, Mitchell AJ, et al. Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection. *Nat Immunol*. 2014;15:45-53.
114. De Filippo K, Dudeck A, Hasenberg M, et al. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. *Blood*. 2013;121:4930-4937.
115. Kim ND, Luster AD. The role of tissue resident cells in neutrophil recruitment. *Trends Immunol*. 2015;36:547-555.
116. Proudfoot AEI, Johnson Z, Bonvin P, Handel TM. Glycosaminoglycan interactions with chemokines add complexity to a complex system. *Pharmaceuticals (Basel)*. 2017;10:70.
117. Bao X, Moseman EA, Saito H, et al. Endothelial heparan sulfate controls chemokine presentation in recruitment of lymphocytes and dendritic cells to lymph nodes. *Immunity*. 2010;33:817-829.
118. Wang L, Fuster M, Sriramarao P, Esko JD. Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nat Immunol*. 2005;6:902-910.
119. Stoler-Barak L, Mousion C, Shezen E, Hatzav M, Sixt M, Alon R. Blood vessels pattern heparan sulfate gradients between their apical and basolateral aspects. *PLoS ONE*. 2014;9:e85699.
120. Yao L, Yago T, Shao B, et al. Elevated CXCL1 expression in gp130-deficient endothelial cells impairs neutrophil migration in mice. *Blood*. 2013;122:3832-3842.

121. Massena S, Christoffersson G, Hjertstrom E, et al. A chemo-tactic gradient sequestered on endothelial heparan sulfate induces directional intraluminal crawling of neutrophils. *Blood*. 2010;116:1924-1931.
122. Middleton J, Neil S, Wintle J, et al. Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell*. 1997;91:385-395.
123. Pruenster M, Mudde L, Bombosi P, et al. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. *Nat Immunol*. 2009;10:101-108.
124. Thiriot A, Perdomo C, Cheng G, et al. Differential DARC/ACKR1 expression distinguishes venular from non-venular endothelial cells in murine tissues. *BMC Biol*. 2017;15:45.
125. Girbl T, Lenn T, Perez L, et al. Distinct Compartmentalization of the chemokines CXCL1 and CXCL2 and the atypical receptor ACKR1 determine discrete stages of neutrophil diapedesis. *Immunity*. 2018;49:1062-1076.e6.
126. Li JL, Lim CH, Tay FW, et al. Neutrophils self-regulate immune complex-mediated cutaneous inflammation through CXCL2. *J Invest Dermatol*. 2016;136:416-424.
127. Sadik CD, Miyabe Y, Sezin T, Luster AD. The critical role of C5a as an initiator of neutrophil-mediated autoimmune inflammation of the joint and skin. *Semin Immunol*. 2018;37:21-29.
128. Kim ND, Chou RC, Seung E, Tager AM, Luster AD. A unique requirement for the leukotriene B4 receptor BLT1 for neutrophil recruitment in inflammatory arthritis. *J Exp Med*. 2006;203:829-835.
129. Chen M, Lam BK, Kanaoka Y, et al. Neutrophil-derived leukotriene B4 is required for inflammatory arthritis. *J Exp Med*. 2006;203:837-842.
130. Chou RC, Kim ND, Sadik CD, et al. Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. *Immunity*. 2010;33:266-278.
131. Miyabe Y, Miyabe C, Murooka TT, et al. Complement C5a receptor is the key initiator of neutrophil adhesion igniting immune complex-induced arthritis. *Sci Immunol*. 2017;2:pii: eaaj2195.
132. Ji H, Ohmura K, Mahmood U, et al. Arthritis critically dependent on innate immune system players. *Immunity*. 2002;16:157-168.
133. Matsumoto I, Maccioni M, Lee DM, et al. How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat Immunol*. 2002;3:360-365.
134. Lee EKS, Gillrie MR, Li L, et al. Leukotriene B4-mediated neutrophil recruitment causes pulmonary capillaritis during lethal fungal sepsis. *Cell Host Microbe*. 2018;23:121-133. e4.
135. Lammermann T, Afonso PV, Angermann BR, et al. Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature*. 2013;498:371-375.
136. Chtanova T, Schaeffer M, Han SJ, et al. Dynamics of neutrophil migration in lymph nodes during infection. *Immunity*. 2008;29:487-496.
137. Majumdar R, Tavakoli Tameh A, Parent CA. Exosomes mediate LTB4 release during neutrophil chemotaxis. *PLoS Biol*. 2016;14:e1002336.
138. Brandt SL, Wang S, DeJani NN, et al. Excessive localized leukotriene B4 levels dictate poor skin host defense in diabetic mice. *JCI Insight*. 2018;3:pii: 120220.
139. McDonald B, Pittman K, Menezes GB, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science*. 2010;330:362-366.
140. Slaba I, Wang J, Kolaczowska E, McDonald B, Lee WY, Kubes P. Imaging the dynamic platelet-neutrophil response in sterile liver injury and repair in mice. *Hepatology*. 2015;62:1593-1605.
141. Honda M, Takeichi T, Hashimoto S, et al. Intravital imaging of neutrophil recruitment reveals the efficacy of FPR1 blockade in hepatic ischemia-reperfusion injury. *J Immunol*. 2017;198:1718-1728.
142. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010;464:104-107.
143. Wang J, Hossain M, Thanabalasuriar A, Gunzer M, Meininger C, Kubes P. Visualizing the function and fate of neutrophils in sterile injury and repair. *Science*. 2017;358:111-116.
144. Sotsios Y, Whittaker GC, Westwick J, Ward SG. The CXC chemokine stromal cell-derived factor activates a Gi-coupled phosphoinositide 3-kinase in T lymphocytes. *J Immunol*. 1999;163:5954-5963.
145. Signoret N, Oldridge J, Pelchen-Matthews A, et al. Phorbol esters and SDF-1 induce rapid endocytosis and down modulation of the chemokine receptor CXCR4. *J Cell Biol*. 1997;139:651-664.
146. Schaeuble K, Hauser MA, Rippl AV, et al. Ubiquitylation of the chemokine receptor CCR7 enables efficient receptor recycling and cell migration. *J Cell Sci*. 2012;125:4463-4474.
147. Mack M, Luckow B, Nelson PJ, et al. Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J Exp Med*. 1998;187:1215-1224.
148. Sauty A, Colvin RA, Wagner L, Rochat S, Spertini F, Luster AD. CXCR3 internalization following T cell-endothelial cell contact: preferential role of IFN-inducible T cell alpha chemoattractant (CXCL11). *J Immunol*. 2001;167:7084-7093.
149. Hecht I, Cahalon L, Hershkoviz R, Lahat A, Franitza S, Lider O. Heterologous desensitization of T cell functions by CCR5 and CXCR4 ligands: inhibition of cellular signaling, adhesion and chemotaxis. *Int Immunol*. 2003;15:29-38.
150. Lin F, Butcher EC. T cell chemotaxis in a simple microfluidic device. *Lab Chip*. 2006;6:1462-1469.
151. Jain NG, Wong EA, Aranyosi AJ, et al. Microfluidic mazes to characterize T-cell exploration patterns following activation in vitro. *Integr Biol (Camb)*. 2015;7:1423-1431.
152. Bousso P, Moreau HD. Functional immunomaging: the revolution continues. *Nat Rev Immunol*. 2012;12:858-864.
153. Krummel MF, Bartumeus F, Gerard A. T cell migration, search strategies and mechanisms. *Nat Rev Immunol*. 2016;16:193-201.
154. Matheu MP, Deane JA, Parker I, Fruman DA, Cahalan MD. Class IA phosphoinositide 3-kinase modulates basal lymphocyte motility in the lymph node. *J Immunol*. 2007;179:2261-2269.
155. Faroudi M, Hons M, Zachacz A, et al. Critical roles for Rac GTPases in T-cell migration to and within lymph nodes. *Blood*. 2010;116:5536-5547.
156. Gerard A, Patino-Lopez G, Beemiller P, et al. Detection of rare antigen-presenting cells through T cell-intrinsic meandering motility, mediated by Myo1g. *Cell*. 2014;158:492-505.
157. Bajenoff M, Egen JG, Koo LY, et al. Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity*. 2006;25:989-1001.
158. Katakai T, Habiro K, Kinashi T. Dendritic cells regulate high-speed interstitial T cell migration in the lymph node via LFA-1/ICAM-1. *J Immunol*. 2013;191:1188-1199.
159. Woolf E, Grigorova I, Sagiv A, et al. Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. *Nat Immunol*. 2007;8:1076-1085.
160. Asperti-Boursin F, Real E, Bismuth G, Trautmann A, Donnadieu E. CCR7 ligands control basal T cell motility within lymph node slices in a phosphoinositide 3-kinase-independent manner. *J Exp Med*. 2007;204:1167-1179.
161. Okada T, Cyster JG. CC chemokine receptor 7 contributes to Gi-dependent T cell motility in the lymph node. *J Immunol*. 2007;178:2973-2978.
162. Hons M, Kopf A, Hauschild R, et al. Chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells. *Nat Immunol*. 2018;19:606-616.
163. Allen CD, Okada T, Cyster JG. Germinal-center organization and cellular dynamics. *Immunity*. 2007;27:190-202.
164. Takeda A, Kobayashi D, Aoi K, et al. Fibroblastic reticular cell-derived lysophosphatidic acid regulates confined intranodal T-cell motility. *eLife*. 2016;5:e10561.

165. Knowlden SA, Capece T, Popovic M, et al. Regulation of T cell motility in vitro and in vivo by LPA and LPA2. *PLoS ONE*. 2014;9:e101655.
166. Calabro S, Liu D, Gallman A, et al. Differential intrasplenic migration of dendritic cell subsets tailors adaptive immunity. *Cell Rep*. 2016;16:2472-2485.
167. Gatto D, Wood K, Caminschi I, et al. The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. *Nat Immunol*. 2013;14:446-453.
168. Yi T, Cyster JG. EBI2-mediated bridging channel positioning supports splenic dendritic cell homeostasis and particulate antigen capture. *eLife*. 2013;2:e00757.
169. Gerner MY, Kastenmuller W, Ifrim I, Kabat J, Germain RN. Histocytometry: a method for highly multiplex quantitative tissue imaging analysis applied to dendritic cell subset microanatomy in lymph nodes. *Immunity*. 2012;37:364-376.
170. Kastenmuller W, Brandes M, Wang Z, Herz J, Egen JG, Germain RN. Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. *Immunity*. 2013;38:502-513.
171. Sung JH, Zhang H, Moseman EA, et al. Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. *Cell*. 2012;150:1249-1263.
172. Brewitz A, Eickhoff S, Dahling S, et al. CD8(+) T cells orchestrate pDC-XCR1(+) dendritic cell spatial and functional cooperativity to optimize priming. *Immunity*. 2017;46:205-219.
173. Hardtke S, Ohl L, Forster R. Balanced expression of CXCR5 and CCR7 on follicular T helper cells determines their transient positioning to lymph node follicles and is essential for efficient B-cell help. *Blood*. 2005;106:1924-1931.
174. Rodda LB, Lu E, Bennett ML, et al. Single-cell RNA sequencing of lymph node stromal cells reveals niche-associated heterogeneity. *Immunity*. 2018;48:1014-1028.e6.
175. Fang V, Chaluvadi VS, Ramos-Perez WD, et al. Gradients of the signaling lipid S1P in lymph nodes position natural killer cells and regulate their interferon-gamma response. *Nat Immunol*. 2017;18:15-25.
176. Kastenmuller W, Torabi-Parizi P, Subramanian N, Lammerrmann T, Germain RN. A spatially-organized multicellular innate immune response in lymph nodes limits systemic pathogen spread. *Cell*. 2012;150:1235-1248.
177. Zhang Y, Roth TL, Gray EE, et al. Migratory and adhesive cues controlling innate-like lymphocyte surveillance of the pathogen-exposed surface of the lymph node. *eLife*. 2016;5:pil18156.
178. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C, Germain RN. Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. *Nature*. 2006;440:890-895.
179. Harris TH, Banigan EJ, Christian DA, et al. Generalized Levy walks and the role of chemokines in migration of effector CD8+ T cells. *Nature*. 2012;486:545-548.
180. Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*. 2004;427:355-360.
181. Lo CG, Xu Y, Proia RL, Cyster JG. Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. *J Exp Med*. 2005;201:291-301.
182. Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol*. 2012;30:69-94.
183. Britschgi MR, Link A, Lissandrin TK, Luther SA. Dynamic modulation of CCR7 expression and function on naive T lymphocytes in vivo. *J Immunol*. 2008;181:7681-7688.
184. Balabanian K, Brotin E, Biajoux V, et al. Proper desensitization of CXCR4 is required for lymphocyte development and peripheral compartmentalization in mice. *Blood*. 2012;119:5722-5730.
185. Arnon TI, Xu Y, Lo C, et al. GRK2-dependent S1PR1 desensitization is required for lymphocytes to overcome their attraction to blood. *Science*. 2011;333:1898-1903.
186. Vroon A, Heijnen CJ, Lombardi MS, et al. Reduced GRK2 level in T cells potentiates chemotaxis and signaling in response to CCL4. *J Leukoc Biol*. 2004;75:901-909.
187. Fong AM, Premont RT, Richardson RM, Yu YR, Lefkowitz RJ, Patel DD. Defective lymphocyte chemotaxis in beta-arrestin2- and GRK6-deficient mice. *Proc Natl Acad Sci USA*. 2002;99:7478-7483.
188. Moratz C, Harrison K, Kehrl JH. Regulation of chemokine-induced lymphocyte migration by RGS proteins. *Methods Enzymol*. 2004;389:15-32.
189. Han SB, Moratz C, Huang NN, et al. Rgs1 and Gnai2 regulate the entrance of B lymphocytes into lymph nodes and B cell motility within lymph node follicles. *Immunity*. 2005;22:343-354.
190. Gibbons DL, Abeler-Dorner L, Raine T, et al. Cutting Edge: regulator of G protein signaling-1 selectively regulates gut T cell trafficking and colitic potential. *J Immunol*. 2011;187:2067-2071.
191. Mackay LK, Rahimpour A, Ma JZ, et al. The developmental pathway for CD103(+)CD8+ tissue-resident memory T cells of skin. *Nat Immunol*. 2013;14:1294-1301.
192. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. *Science*. 2013;341:1483-1488.
193. Druzd D, Matveeva O, Ince L, et al. Lymphocyte circadian clocks control lymph node trafficking and adaptive immune responses. *Immunity*. 2017;46:120-132.
194. Scheiermann C, Gibbs J, Ince L, Loudon A. Clocking in to immunity. *Nat Rev Immunol*. 2018;18:423-437.
195. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol*. 2014;14:377-391.
196. Takahama Y. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol*. 2006;6:127-135.
197. James KD, Jenkinson WE, Anderson G. T-cell egress from the thymus: should I stay or should I go? *J Leukoc Biol*. 2018;104:275-284.
198. Girard JP, Moussion C, Forster R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nat Rev Immunol*. 2012;12:762-773.
199. Misslitz A, Pabst O, Hintzen G, et al. Thymic T cell development and progenitor localization depend on CCR7. *J Exp Med*. 2004;200:481-491.
200. Ueno T, Saito F, Gray DH, et al. CCR7 signals are essential for cortex-medulla migration of developing thymocytes. *J Exp Med*. 2004;200:493-505.
201. Kozai M, Kubo Y, Katakai T, et al. Essential role of CCL21 in establishment of central self-tolerance in T cells. *J Exp Med*. 2017;214:1925-1935.
202. Link A, Vogt TK, Favre S, et al. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol*. 2007;8:1255-1265.
203. Plotkin J, Prockop SE, Lepique A, Petrie HT. Critical role for CXCR4 signaling in progenitor localization and T cell differentiation in the postnatal thymus. *J Immunol*. 2003;171:4521-4527.
204. Benz C, Heinzel K, Bleul CC. Homing of immature thymocytes to the subcapsular microenvironment within the thymus is not an absolute requirement for T cell development. *Eur J Immunol*. 2004;34:3652-3663.
205. Mingueneau M, Kreslavsky T, Gray D, et al. The transcriptional landscape of alphabeta T cell differentiation. *Nat Immunol*. 2013;14:619-632.
206. Tomura M, Yoshida N, Tanaka J, et al. Monitoring cellular movement in vivo with photoconvertible fluorescence protein "Kaede" transgenic mice. *Proc Natl Acad Sci USA*. 2008;105:10871-10876.
207. Mandl JN, Liou R, Klauschen F, et al. Quantification of lymph node transit times reveals differences in antigen surveillance



- strategies of naive CD4+ and CD8+ T cells. *Proc Natl Acad Sci USA*. 2012;109:18036-18041.
208. Pham TH, Okada T, Matloubian M, Lo CG, Cyster JG. S1P1 receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity*. 2008;28:122-133.
209. Qi H. T follicular helper cells in space-time. *Nat Rev Immunol*. 2016;16:612-625.
210. Buchholz VR, Graf P, Busch DH. The origin of diversity: studying the evolution of multi-faceted CD8+ T cell responses. *Cell Mol Life Sci*. 2012;69:1585-1595.
211. Gerritsen B, Pandit A. The memory of a killer T cell: models of CD8(+) T cell differentiation. *Immunol Cell Biol*. 2016;94:236-241.
212. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (\*). *Annu Rev Immunol*. 2010;28:445-489.
213. Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol*. 2011;29:621-663.
214. O'Garra A, McEvoy LM, Zlotnik A. T-cell subsets: chemokine receptors guide the way. *Curr Biol*. 1998;8:R646-R649.
215. Vinuesa CG, Cyster JG. How T cells earn the follicular rite of passage. *Immunity*. 2011;35:671-680.
216. Groom JR, Richmond J, Murooka TT, et al. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. *Immunity*. 2012;37:1091-1103.
217. Turner JE, Paust HJ, Steinmetz OM, et al. CCR6 recruits regulatory T cells and Th17 cells to the kidney in glomerulonephritis. *J Am Soc Nephrol*. 2010;21:974-985.
218. Paust HJ, Riedel JH, Krebs CF, et al. CXCR3+ regulatory T cells control TH1 responses in crescentic GN. *J Am Soc Nephrol*. 2016;27:1933-1942.
219. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat Immunol*. 2009;10:595-602.
220. Wang Y, Su MA, Wan YY. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity*. 2011;35:337-348.
221. Chung Y, Tanaka S, Chu F, et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat Med*. 2011;17:983-988.
222. Wurbel MA, Malissen B, Campbell JJ. Complex regulation of CCR9 at multiple discrete stages of T cell development. *Eur J Immunol*. 2006;36:73-81.
223. Carramolino L, Zaballos A, Kremer L, et al. Expression of CCR9 beta-chemokine receptor is modulated in thymocyte differentiation and is selectively maintained in CD8(+) T cells from secondary lymphoid organs. *Blood*. 2001;97:850-857.
224. Matloubian M, David A, Engel S, Ryan JE, Cyster JG. A transmembrane CXC chemokine is a ligand for HIV-coreceptor Bonzo. *Nat Immunol*. 2000;1:298-304.
225. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature*. 2004;427:154-159.
226. Stoll S, Delon J, Brotz TM, Germain RN. Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science*. 2002;296:1873-1876.
227. Bousso P, Robey E. Dynamics of CD8+ T cell priming by dendritic cells in intact lymph nodes. *Nat Immunol*. 2003;4:579-585.
228. Miller MJ, Wei SH, Parker I, Cahalan MD. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. *Science*. 2002;296:1869-1873.
229. Eickhoff S, Brewitz A, Gerner MY, et al. Robust anti-viral immunity requires multiple distinct T cell-dendritic cell interactions. *Cell*. 2015;162:1322-1337.
230. Jusforgues-Saklani H, Uhl M, Blachere N, et al. Antigen persistence is required for dendritic cell licensing and CD8+ T cell cross-priming. *J Immunol*. 2008;181:3067-3076.
231. Castellino F, Germain RN. Cooperation between CD4+ and CD8+ T cells: when, where, and how. *Annu Rev Immunol*. 2006;24:519-540.
232. Hugues S, Scholer A, Boissonnas A, et al. Dynamic imaging of chemokine-dependent CD8+ T cell help for CD8+ T cell responses. *Nat Immunol*. 2007;8:921-930.
233. Hor JL, Whitney PG, Zaid A, Brooks AG, Heath WR, Mueller SN. Spatiotemporally distinct interactions with dendritic cell subsets facilitates CD4+ and CD8+ T cell activation to localized viral infection. *Immunity*. 2015;43:554-565.
234. Kurachi M, Kurachi J, Suenaga F, et al. Chemokine receptor CXCR3 facilitates CD8(+) T cell differentiation into short-lived effector cells leading to memory degeneration. *J Exp Med*. 2011;208:1605-1620.
235. Hu JK, Kagari T, Clingan JM, Matloubian M. Expression of chemokine receptor CXCR3 on T cells affects the balance between effector and memory CD8 T-cell generation. *Proc Natl Acad Sci USA*. 2011;108:E118-E127.
236. de Lemos C, Christensen JE, Nansen A, et al. Opposing effects of CXCR3 and CCR5 deficiency on CD8+ T cell-mediated inflammation in the central nervous system of virus-infected mice. *J Immunol*. 2005;175:1767-1775.
237. Kohlmeier JE, Reiley WW, Perona-Wright G, et al. Inflammatory chemokine receptors regulate CD8(+) T cell contraction and memory generation following infection. *J Exp Med*. 2011;208:1621-1634.
238. Botzcher JP, Beyer M, Meissner F, et al. Functional classification of memory CD8(+) T cells by CX3CR1 expression. *Nat Commun*. 2015;6:8306.
239. Nishimura M, Umehara H, Nakayama T, et al. Dual functions of fractalkine/CX3C ligand 1 in trafficking of perforin+/granzyme B+ cytotoxic effector lymphocytes that are defined by CX3CR1 expression. *J Immunol*. 2002;168:6173-6180.
240. Gerlach C, Moseman EA, Loughhead SM, et al. The chemokine receptor CX3CR1 defines three antigen-experienced CD8 T cell subsets with distinct roles in immune surveillance and homeostasis. *Immunity*. 2016;45:1270-1284.
241. Mantovani A. Redundancy and robustness versus division of labour and specialization in innate immunity. *Semin Immunol*. 2018;36:28-30.
242. Gouwy M, Struyf S, Proost P, Van Damme J. Synergy in cytokine and chemokine networks amplifies the inflammatory response. *Cytok Growth Fact Rev*. 2005;16:561-580.
243. Stanford MM, Issekutz TB. The relative activity of CXCR3 and CCR5 ligands in T lymphocyte migration: concordant and disparate activities in vitro and in vivo. *J Leukoc Biol*. 2003;74:791-799.
244. Sancho D, Reis e Sousa C. Signaling by myeloid C-type lectin receptors in immunity and homeostasis. *Annu Rev Immunol*. 2012;30:491-529.
245. Wu J, Chen ZJ. Innate immune sensing and signaling of cytosolic nucleic acids. *Annu Rev Immunol*. 2014;32:461-488.
246. Pearce EJ, Everts B. Dendritic cell metabolism. *Nat Rev Immunol*. 2015;15:18-29.
247. Steinman RM. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol*. 2012;30:1-22.
248. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol*. 2002;20:621-667.
249. Worbs T, Hammerschmidt SI, Forster R. Dendritic cell migration in health and disease. *Nat Rev Immunol*. 2017;17:30-48.
250. Gerner MY, Casey KA, Kastenmuller W, Germain RN. Dendritic cell and antigen dispersal landscapes regulate T cell immunity. *J Exp Med*. 2017;214:3105-3122.
251. Guilliams M, van de Laar L. A Hitchhiker's guide to myeloid cell subsets: practical implementation of a novel mononuclear phagocyte classification system. *Front Immunol*. 2015;6:406.
252. Swiecki M, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. *Nat Rev Immunol*. 2015;15:471-485.

253. Dominguez PM, Ardavin C. Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. *Immunol Rev*. 2010;234:90-104.
254. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol*. 2014;14:392-404.
255. Snyderman R, Goetzl EJ. Molecular and cellular mechanisms of leukocyte chemotaxis. *Science*. 1981;213:830-837.
256. Haessler U, Pisano M, Wu M, Swartz MA. Dendritic cell chemotaxis in 3D under defined chemokine gradients reveals differential response to ligands CCL21 and CCL19. *Proc Natl Acad Sci USA*. 2011;108:5614-5619.
257. Helft J, Bottcher J, Chakravarty P, et al. GM-CSF Mouse bone marrow cultures comprise a heterogeneous population of CD11c(+)MHCII(+) macrophages and dendritic cells. *Immunity*. 2015;42:1197-1211.
258. Forster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol*. 2008;8:362-371.
259. Purvanov V, Matti C, Samson GPB, Kindinger I, Legler DF. Fluorescently tagged CCL19 and CCL21 to monitor CCR7 and ACKR4 functions. *Int J Mol Sci*. 2018;19:pii: E3876.
260. Jorgensen AS, Adogamhe PE, Laufer JM, et al. CCL19 with CCL21-tail displays enhanced glycosaminoglycan binding with retained chemotactic potency in dendritic cells. *J Leukoc Biol*. 2018;104:401-411.
261. Hjorto GM, Larsen O, Steen A, et al. Differential CCR7 Targeting in dendritic cells by three naturally occurring CC-chemokines. *Front Immunol*. 2016;7:568.
262. Hauser MA, Legler DF. Common and biased signaling pathways of the chemokine receptor CCR7 elicited by its ligands CCL19 and CCL21 in leukocytes. *J Leukoc Biol*. 2016;99:869-882.
263. Otero C, Groettrup M, Legler DF. Opposite fate of endocytosed CCR7 and its ligands: recycling versus degradation. *J Immunol*. 2006;177:2314-2323.
264. Jorgensen AS, Rosenkilde MM, Hjorto GM. Biased signaling of G protein-coupled receptors - From a chemokine receptor CCR7 perspective. *Gen Comp Endocrinol*. 2018;258:4-14.
265. Zidar DA, Violin JD, Whalen EJ, Lefkowitz RJ. Selective engagement of G protein coupled receptor kinases (GRKs) encodes distinct functions of biased ligands. *Proc Natl Acad Sci USA*. 2009;106:9649-9654.
266. Lammerrmann T, Bader BL, Monkley SJ, et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature*. 2008;453:51-55.
267. Petrie Aronin CE, Zhao YM, Yoon JS, et al. Migrating myeloid cells sense temporal dynamics of chemoattractant concentrations. *Immunity*. 2017;47:862-874.e3.
268. Wang Y, Irvine DJ. Convolution of chemoattractant secretion rate, source density, and receptor desensitization direct diverse migration patterns in leukocytes. *Integr Biol*. 2013;5:481-494.
269. Schwarz J, Bierbaum V, Vaahomeri K, et al. Dendritic cells interpret haptotactic chemokine gradients in a manner governed by signal-to-noise ratio and dependent on GRK6. *Curr Biol*. 2017;27:1314-1325.
270. Schwarz J, Bierbaum V, Merrin J, et al. A microfluidic device for measuring cell migration towards substrate-bound and soluble chemokine gradients. *Sci Rep*. 2016;6:36440.
271. Hansen M, Met O, Larsen NB, et al. Autocrine CCL19 blocks dendritic cell migration toward weak gradients of CCL21. *Cytotherapy*. 2016;18:1187-1196.
272. Kabashima K, Shiraishi N, Sugita K, et al. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. *Am J Pathol*. 2007;171:1249-1257.
273. Legler DF, Krause P, Scandella E, Singer E, Groettrup M. Prostaglandin E2 is generally required for human dendritic cell migration and exerts its effect via EP2 and EP4 receptors. *J Immunol*. 2006;176:966-973.
274. Czeloth N, Bernhardt G, Hofmann F, Genth H, Forster R. Sphingosine-1-phosphate mediates migration of mature dendritic cells. *J Immunol*. 2005;175:2960-2967.
275. Lindquist RL, Shakhar G, Dudziak D, et al. Visualizing dendritic cell networks in vivo. *Nat Immunol*. 2004;5:1243-1250.
276. Hashimoto M, Yanagisawa H, Minagawa S, et al. TGF-beta-dependent dendritic cell chemokinesis in murine models of airway disease. *J Immunol*. 2015;195:1182-1190.
277. Umemoto E, Otani K, Ikono T, et al. Constitutive plasmacytoid dendritic cell migration to the splenic white pulp is cooperatively regulated by CCR7- and CXCR4-mediated signaling. *J Immunol*. 2012;189:191-199.
278. Seth S, Oberdorfer L, Hyde R, et al. CCR7 essentially contributes to the homing of plasmacytoid dendritic cells to lymph nodes under steady-state as well as inflammatory conditions. *J Immunol*. 2011;186:3364-3372.
279. Tal O, Lim HY, Gurevich I, et al. DC mobilization from the skin requires docking to immobilized CCL21 on lymphatic endothelium and intralymphatic crawling. *J Exp Med*. 2011;208:2141-2153.
280. Russo E, Teixeira A, Vaahomeri K, et al. Intralymphatic CCL21 promotes tissue egress of dendritic cells through afferent lymphatic vessels. *Cell Rep*. 2016;14:1723-1734.
281. Braun A, Worbs T, Moschovakis GL, et al. Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration. *Nat Immunol*. 2011;12:879-887.
282. Randolph GJ, Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat Rev Immunol*. 2005;5:617-628.
283. Ardouin L, Luche H, Chelbi R, et al. Broad and largely concordant molecular changes characterize tolerogenic and immunogenic dendritic cell maturation in thymus and periphery. *Immunity*. 2016;45:305-318.
284. Baratin M, Foray C, Demaria O, et al. Homeostatic NF-kappaB signaling in steady-state migratory dendritic cells regulates immune homeostasis and tolerance. *Immunity*. 2015;42:627-639.
285. Yanagihara S, Komura E, Nagafune J, Watarai H, Yamaguchi Y. EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. *J Immunol*. 1998;161:3096-3102.
286. Sallusto F, Schaerli P, Loetscher P, et al. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol*. 1998;28:2760-2769.
287. Ohl L, Mohaupt M, Czeloth N, et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity*. 2004;21:279-288.
288. Bajenoff M, Granjeaud S, Guerder S. The strategy of T cell antigen-presenting cell encounter in antigen-draining lymph nodes revealed by imaging of initial T cell activation. *J Exp Med*. 2003;198:715-724.
289. Kissenpfennig A, Henri S, Dubois B, et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity*. 2005;22:643-654.
290. Woodruff MC, Heesters BA, Herndon CN, et al. Trans-nodal migration of resident dendritic cells into medullary interfollicular regions initiates immunity to influenza vaccine. *J Exp Med*. 2014;211:1611-1621.
291. Byers MA, Calloway PA, Shannon L, et al. Arrestin 3 mediates endocytosis of CCR7 following ligation of CCL19 but not CCL21. *J Immunol*. 2008;181:4723-4732.
292. Moore CA, Milano SK, Benovic JL. Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol*. 2007;69:451-482.
293. Cai Y, Yang C, Yu X, et al. Deficiency of beta-Arrestin 2 in dendritic cells contributes to autoimmune diseases. *J Immunol*. 2019;202:407-420.



294. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. *Nat Rev Immunol.* 2019;19:89-103.
295. Britschgi MR, Favre S, Luther SA. CCL21 is sufficient to mediate DC migration, maturation and function in the absence of CCL19. *Eur J Immunol.* 2010;40:1266-1271.
296. Gaiser MR, Lammermann T, Feng X, et al. Cancer-associated epithelial cell adhesion molecule (EpCAM; CD326) enables epidermal Langerhans cell motility and migration in vivo. *Proc Natl Acad Sci USA.* 2012;109:E889-E897.
297. Ouwehand K, Santegoets SJ, Bruynzeel DP, Scheper RJ, de Gruijl TD, Gibbs S. CXCL12 is essential for migration of activated Langerhans cells from epidermis to dermis. *Eur J Immunol.* 2008;38:3050-3059.
298. Ouwehand K, Scheper RJ, de Gruijl TD, Gibbs S. Epidermis-to-dermis migration of immature Langerhans cells upon topical irritant exposure is dependent on CCL2 and CCL5. *Eur J Immunol.* 2010;40:2026-2034.
299. Stutte S, Quast T, Gerbitzki N, et al. Requirement of CCL17 for CCR7- and CXCR4-dependent migration of cutaneous dendritic cells. *Proc Natl Acad Sci USA.* 2010;107:8736-8741.
300. Sokol CL, Camire RB, Jones MC, Luster AD. The chemokine receptor CCR8 promotes the migration of dendritic cells into the lymph node parenchyma to initiate the allergic immune response. *Immunity.* 2018;49:449-463.e6.
301. Yabe R, Shimizu K, Shimizu S, et al. CCR8 regulates contact hypersensitivity by restricting cutaneous dendritic cell migration to the draining lymph nodes. *Int Immunol.* 2015;27:169-181.
302. Qu C, Edwards EW, Tacke F, et al. Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. *J Exp Med.* 2004;200:1231-1241.
303. Teijeira A, Russo E, Halin C. Taking the lymphatic route: dendritic cell migration to draining lymph nodes. *Semin Immunopathol.* 2014;36:261-274.
304. Leon B, Ballesteros-Tato A, Browning JL, Dunn R, Randall TD, Lund FE. Regulation of T(H)2 development by CXCR5+ dendritic cells and lymphotoxin-expressing B cells. *Nat Immunol.* 2012;13:681-690.
305. Cook DN, Prosser DM, Forster R, et al. CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity.* 2000;12:495-503.
306. Shreedhar VK, Kelsall BL, Neutra MR. Cholera toxin induces migration of dendritic cells from the subepithelial dome region to T- and B-cell areas of Peyer's patches. *Infect Immun.* 2003;71:504-509.
307. Natsuaki Y, Egawa G, Nakamizo S, et al. Perivascular leukocyte clusters are essential for efficient activation of effector T cells in the skin. *Nat Immunol.* 2014;15:1064-1069.
308. Iannacone M, Moseman EA, Tonti E, et al. Subcapsular sinus macrophages prevent CNS invasion on peripheral infection with a neurotropic virus. *Nature.* 2010;465:1079-1083.
309. Roberts EW, Broz ML, Binnewies M, et al. Critical role for CD103(+)/CD141(+) dendritic cells bearing CCR7 for tumor antigen trafficking and priming of T cell immunity in melanoma. *Cancer Cell.* 2016;30:324-336.
310. Broz ML, Binnewies M, Boldajipour B, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell.* 2014;26:638-652.
311. Bottcher JP, Bonavita E, Chakravarty P, et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell.* 2018;172:1022-1037.e14.
312. Schulz O, Hammerschmidt SI, Moschovakis GL, Forster R. Chemokines and chemokine receptors in lymphoid tissue dynamics. *Annu Rev Immunol.* 2016;34:203-242.
313. Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol.* 2007;25:787-820.
314. Sarris M, Olekhnovitch R, Bousso P. Manipulating leukocyte interactions in vivo through optogenetic chemokine release. *Blood.* 2016;127:e35-e41.

**How to cite this article:** Lämmermann T, Kastenmüller W. Concepts of GPCR-controlled navigation in the immune system. *Immunol Rev.* 2019;289:205-231. <https://doi.org/10.1111/imr.12752>