ABSTRACT

Chemoattractants are pivotal mediators of host defense, orchestrating the recruitment of immune cells into sites of infection and inflammation. Chemoattractants display vast chemical diversity and include bioactive lipids, proteolytic fragments of serum proteins, and chemokines (chemotactic cytokines). All chemoattractants induce chemotaxis by activating seven-transmembrane-spanning GPCRs expressed on immune cells, establishing the concept that all chemoattractants are related in function. However, although chemoattractants have overlapping functions in vitro, recent in vivo data have revealed that they function, in many cases, nonredundantly in vivo. The chemically diverse nature of chemoattractants contributes to the fine control of leukocyte trafficking in vivo, with sequential chemoattractant use guiding immune cell recruitment into inflammatory sites. Lipid mediators frequently function as initiators of leukocyte recruitment, attracting the first immune cells into tissues. These initial responding immune cells produce cytokines locally, which in turn, induce the local release of chemokines. Local chemokine production then markedly amplifies subsequent waves of leukocyte recruitment. These new discoveries establish a paradigm for leukocyte recruitment, described as lipid-chemokine cascades—as a driving force in the effector phase of immune responses. J. Leukoc. Biol. 91: 207–215; 2012.

IMMUNE CELL RECRUITMENT GOVERNED BY LIPID MEDIATOR-CHEMOKINE-CYTOKINE CASCADES

Swift recruitment of immune cells to peripheral tissues is a hallmark of effective host defense. Recruitment of immune cells into healthy tissues, in contrast, contributes importantly to the pathogenesis of numerous autoimmune and inflammatory diseases, such as acute lung and spinal injury, RA, and asthma. Dysregulated recruitment of immune cells into peripheral tissue leads to chronic disease and irreversible tissue damage [1–8]. The recruitment of immune cells into peripheral tissues is choreographed by chemoattractants, a chemically diverse group of molecular guidance signals, including lipid mediators (e.g., LTB4), proteolytic fragments of serum proteins (e.g., complement components C3a and C5a), tissue proteins, such as collagen peptides (e.g., proline-glycine-proline), and chemotactic cytokines (e.g., chemokines). Chemoattractants induce directed cell migration by activating seven-transmembrane-spanning GPCRs, expressed on the surface of immune cells [9]. In vitro, chemoattractants exhibit largely overlapping functions, and pathological specimens often harbor an array of diverse chemoattractants. This implies that chemoattractants may play redundant roles in the recruitment of immune cells and thus, in host defense and pathological processes. Seminal discoveries made over the last decade, however, suggest that in vivo, chemoattractants cooperate temporally and spatially to finely control the movement of immune cells out of the bone marrow, into the circulation, and then into sites of inflammation within peripheral tissues [10–13]. These nonredundant roles for individual chemoattractants arise from differences in their temporal and spatial production, differences in their biophysical properties, as well as in some cases, differences in their effects on target cells [10, 11]. Moreover, differences in the responsiveness of immune cells to different classes of chemoattractants may play a significant role in the regulation of chemoattractant cascades. When neutrophils, for instance, are exposed to more than one chemotactic guidance signal at the same time, they prioritize between “intermediate target” (e.g., chemokines and LTB4) and “end-target” (e.g., C5a, C3a, and N-formyl peptides) chemoattractants and follow the end-target signal. The distinction between intermediate and end-target

Abbreviations: 5-LO=5-lipoxygenase, 5-oxo-ETE=5-oxo-6,8,11,14-eicosatetraenoic acid, 12-HHT=12(S)-hydroxyheptadeca-5Z,8E,10E-trienoic acid, BLT1/2=leukotriene B4 receptor 1/2, DP1/2=PGD2 receptor type 1/2, FLS=fibrolast-like synovocyte, GPCR=G protein-coupled receptor, LTA4H=leukotriene A4-hydrolase, LTB4=leukotriene B4, Ltb4r1+/− and Ltb4r1−/− = leukotriene B4 receptor 1-deficient and WT mice, respectively, mDC=myeloid DC, PAF=platelet-activating factor, PAFR=platelet-activating factor’s own seven-transmembrane-spanning receptor, PGDS=PGD-synthase, RA=rheumatoid arthritis

1. Correspondence: Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy, and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. E-mail: aluster@mgh.harvard.edu
signals has been suggested to depend on the phosphorylation level of p38 MAPK within the cell, as only end-target signals phosphorylate p38 MAPK, and additionally, PI3K phosphatase and tensin homologue are required for this distinction [14–16]. The concept of intermediate target and end-target chemotaxtants has been deduced from in vitro observations and still awaits validation in vivo. Other regulatory mechanisms, which may help to prioritize between opposing guidance signals, include “priming” and “chemoattractant receptor cross-desensitization”. Here, chemotaxtactant ligand binding positively (priming) or negatively (desensitization) shifts the responsiveness of the cell to subsequent signals from other chemotaxtactants [17]. However, the overall importance of these mechanisms in vivo has not been elucidated.

A common principle of sequential chemotaxtactant cascades has emerged with important roles for lipid mediators, particularly eicosanoids, as initiators of leukocyte recruitment kindling the cascade before cytokines induce the production of chemokines, which further amplify the recruitment of immune cells (Fig. 1). This principle may be related to the ultra-rapid production of lipid mediators, whereas the expression of chemokines is usually slower, controlled at the transcriptional and translational levels, and therefore, requires more time before they can be released. However, chemokines have a longer half-life and have the ability to act at far greater distances than lipid mediators [18–21], enabling them to amplify and sustain the recruitment of immune cells for longer periods of time.

**BIOCHEMISTRY OF EICOSANOIDs AND THEIR RECEPTORS**

Eicosanoids are the most important class of lipid mediators involved in the recruitment of immune cells. They constitute a large family of diverse lipid mediators derived from arachidonic acid, a 20-carbon polyunsaturated fatty acid. Arachidonic acid is usually kept esterified to membrane phospholipids, until mobilized for biosynthesis by members of the PLA2 family, hydrolyzing the ester bond in the sn-2 position of phospholipids to yield free arachidonic acid and lysophospholipids [22]. The activity of PLA2 is tightly controlled and is a key checkpoint in the regulation of eicosanoid biosynthesis. PLA2 activity depends on its phosphorylation status, which is regulated, in part, by PKC and on calcium-dependent translocation of the enzyme from the cytosol to cell membranes [23]. Bioactive eicosanoids are not stored in the cell but rapidly produced de novo upon activation. Diverse signals can induce the release of LTB4, depending on the cell type and the state of activation of the cell, and include immune complexes, antigens, cytokines, complement, and microbes [24]. The biosynthesis of eicosanoids from arachidonic acid always requires at least one oxygenation step catalyzed by COX, lipoxygenases, or cytochrome P450 mono-oxidases. We will focus our discussion on two specific eicosanoids that have been shown to play important roles in the initiation of chemotaxtactant cascades in vivo: LTB4 and PGD2.

The first step in the biosynthesis of LTs is the conversion of arachidonic acid to LTA4 by 5-LO [24], which catalyzes a two-step reaction with 5-hydroperoxecosatetraenoic acid as an intermediate, which is instantaneously, further converted to LTA4 [25, 26]. 5-LO translocates from the cytosol to the nuclear membrane to associate with the 5-LO-activating protein, which presents bound arachidonic acid to 5-LO [27, 28]. LTA4 serves as substrate for the generation of all bioactive LTs. For the generation of LTB4, LTA4 is further converted by LTA4H, which dehydrates LTA4 to the final product, LTB4. The activity of 5-LO and LTA4H is short-lived with both enzymes prone to suicide inactivation [29–31]. Although 5-LO expression is restricted to myeloid cells, including neutrophils, eosinophils, monocytes/macrophages, and mast cells, LTA4H is widely expressed in hematopoietic and stromal tissues [32]. Thus, although LTB4 biosynthesis ultimately depends on the activity of myeloid cells, other cell types can participate in the generation of LTB4 in a transcellular manner by converting the myeloid cell-derived LTA4 to LTB4 [33, 34]. As LTA4H is subject to suicide inactivation, the transcellular pathway of LTB4 production may have biological significance in the amplification and sustainment of acute immune responses [33].

LTB4 can bind to two highly conserved GPCRs, BLT1 and BLT2 [35–37]. LTB4 displays high affinity for BLT1 (Kd ~0.5 nM), whereas the affinity to BLT2 is considerably lower (Kd ~23 nM) [38]. Activation of either increases intracellular Ca2+ levels and decreases cAMP levels [24]. Notably, 12-HHT, a product of COX-1, has recently been described as a second ligand for BLT2 with a higher affinity for this receptor than LTB4 [39]. The biological significance of LTB4 or 12-HHT binding to BLT2 in vivo, however, remains unknown.

BLT1 is mainly expressed on immune cells, including neutrophils, eosinophils, basophils, monocytes/macrophages, and DCs, as well as effector CD4+ and CD8+ T cells [37, 40–47]. It has also been found on some nonhematopoietic cells, such as synovial fibroblast-like cells [33]. Activation of BLT1 induces chemotaxis and firm adhesion of leukocytes [46]. Depending on the cell type, it can exert additional effects. In neutrophils, for instance, it induces degranulation and ROS production and inhibits apoptosis, whereas in monocytes/macrophages, it induces phagocytosis, CCR2, and MCP-1 expression [48–50].

In humans, BLT2 is widely expressed on hematopoietic and nonhematopoietic cells. In contrast, in mice, BLT2 expression is

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**Figure 1. General principle of “lipid-cytokine-chemokine cascades” in the initiation of inflammatory responses.** Upon stimulation, the tissue sentinel leukocyte immediately begins to release LTB4 and possibly additional lipid mediators. In turn, the first effector immune cells are recruited into the inflammatory site by lipid mediators. These first recruited immune cells release proinflammatory cytokines, which activate tissue resident cells. Activated tissue resident cells release chemokines, which amplify immune cell recruitment into the inflammatory site.
restricted to few cell types, such as mast cells and keratinocytes [39, 51]. Knowledge about the function and biological significance of BLT2 is scarce. Recently, however, nonredundant roles for BLT1 and BLT2 in inflammatory arthritis in mice have been shown, ascribing a role of BLT2 expression on hematopoietic cells to inflammatory processes amplifying arthritis [52].

PGs are generated from arachidonic acid by the COX pathway. COX-1 and COX-2 convert arachidonic acid, first to PGH2, which is further converted to the respective end product by the activity of specific synthases [53]. PGDS accordingly convert PGH2 to PGD2. Two isoforms of PGDS have been defined: lipocalin-type PGDS, expressed in the CNS and the male reproductive system, and hematopoietic PGDS, expressed in immune cells, such as mast cells, TH2 cells, macrophages, and DCs [54]. PGD2 is the major PG released by mast cells after activation by IgE, and mast cells are the major source of PGD2 in vivo [55, 56].

There are two receptors for PGD2: DP1 and DP2; the latter was first named chemoattractant receptor-homologous molecule expressed on Th2 cells [57]. Both are seven-transmembrane-domain GPCRs but engage different signaling pathways and are phylogenetically distinctly related [53, 58]. DP1 is expressed on hematopoietic and nonhematopoietic cells. Among the hematopoietic cells, eosinophils, basophils, monocytes, DCs, T41, and T112 cells express DP1. Binding of PGD2 to DP1 activates Gs proteins, which in turn, stimulate adenyl cyclase and elevation of intracellular cAMP and Ca2+ levels in the cell [59]. DP2, in contrast, activates Gi proteins, which inhibit adenyl cyclase, reduce cAMP levels, and activate PLCβ to elevate inositol triphosphatase and DAG levels in the cell. DP2 is expressed exclusively on immune cells, especially on T112 cells in humans, but expression on cytotoxic T cells, eosinophils, basophils, mast cells, and monocytes has been described [13, 58, 60]. The function of DP2 is still controversial and may include pro- and anti-inflammatory effects. But nevertheless, like DP1, DP2 has been implicated in the pathogenesis of allergic diseases [58, 61, 62].

Here, we focus primarily on LTB4 and to a lesser extent, on PDGAs, as the in vivo role of these lipid mediators in chemoattractant cascades has been the best characterized. In vitro data, however, suggest that other eicosanoids may similarly participate in chemoattractant cascades as a result of their rapid production following diverse stimuli and their strong activating and chemotactic effect on leukocytes. One notable example of other lipid mediators is PAF, which is a potent proinflammatory phospholipid exerting pleotropic effects on immune cells. It is biosynthesized from phosphatidylcholine, converted first by PLAc2 and subsequently, by lyso-PAF-acetyltransferase [63]. It binds to PAFR and activates several proinflammatory signaling pathways, including NF-kB and PKC [64]. PAFR is widely expressed on immune cells and has been implicated in the pathogenesis of numerous autoimmune diseases, as well as in systemic inflammatory responses [64, 65]. PAF and LTB4 are often concomitantly produced, and recently, it has been shown that both mediators act in concert to recruit neutrophils upon stimulation with diverse TLR ligands in vitro and in vivo [66]; hence, in some settings, both lipid mediators may be required to initiate chemoattractant cascades.

Additionally, 5-LO products other than LTB4, such as 5-oxoETE, may participate in chemoattractant cascades. 5-oxo-ETE is biosynthesized by monocytes, neutrophils, basophils, and eosinophils and exerts chemotactic and activating effects upon these same cells, most pronounced upon eosinophils, via its highly selective OXE receptor [67, 68]. 5-oxo-ETE can also synergize with chemokines, such as MCP-1, MCP-3, RANTES, and eotaxin [69]. Although 5-oxo-ETE has been surmised to play a role in asthma, cancer, and cardiovascular diseases [70], up until now, there is no clear evidence for its pathophysiological role in these disease processes in vivo or its participation in chemoattractant cascades in vivo.

**LIPID-CHEMOKINE-CYTOKINE CASCADE IN ALLERGIC ASTHMA**

**Lipid initiation**

Asthma is a complex inflammatory disease with diverse immune cells, among them TH2 cells, eosinophils, basophils, and neutrophils, infiltrating the airway and lung parenchyma [71]. LTB4 and PGD2 are elevated in the airways, BALs, and exhaled breath condensates of asthma patients [72–79]. Intriguingly, data from murine models of asthma suggest that both play a pivotal role in the generation of asthma. Thus, in the active immunization model of allergic airway inflammation, LTB4-induced T cell recruitment mediated by BLT1 is crucial in the early pathogenesis of asthma [41]. Herein, BLT1 is particularly required for the initial entry of TH2 cells into the airways. Mast cells are presumably the major source of LTB4 (although this has not been demonstrated in vivo yet), as mast cell-derived LTB4 can induce the migration of TH2 cells into the airways after challenge with antigen [82], and intriguingly, data from murine models of asthma suggest that both play a pivotal role in the generation of effector T cells in that model not only inhibited the recruitment of effector T cells but also improved overall survival after lung transplantation. In support of a pivotal role of BLT1 in the generation of allergic asthma, BLT1 was shown to be required for the induction of the Th1 cell response in other models of allergic pulmonary inflammation associated with airway hyper-responsiveness [81]. Thus, in the absence of BLT1, the levels of TH1 cytokines and eosinophilia in the airways were reduced, and the development of airway hyper-responsiveness was prevented [81]. Furthermore, BLT1 also contributes to directly recruit neutrophils and eosinophils into the airways after challenge with antigen [82], and intriguingly, LTB4 can also recruit mast cell progenitors [83]. Therefore, a positive-feedback loop in the generation of asthma, in which mast cell-derived LTB4 recruits additional mast cell progenitors to the lung to amplify asthma, is conceivable.

Studies using genetically deficient mice also suggest an important role for PGD2 in T cell trafficking and the pathogenesis of asthma [84]. In an active immunization model using OVA aerosol allergen challenge, DP1-deficient mice showed a specific reduction of TH2 cell recruitment into the lung.

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whereas at the same time, the recruitment of T112 cells was unaltered. Consequently to the reduction of T112 cell recruitment, the levels of the T112 cytokines IL-4, IL-5, and IL-13 were decreased, which in turn, decreased the recruitment of eosinophils, mucus hypersecretion, and airway hyper-responsiveness. Conversely, overexpression of PGDS with subsequent exuberant production of PGD2 displayed exacerbated disease with increased numbers of T112 cells and eosinophils recruited into the lung [85]. In humans, DP2 is highly expressed on T112 cells and likely exerts a direct effect on T112 cell recruitment into the allergic lung [86]. In mice, DP2 is not expressed on T112 cells, and PGD2 was shown to recruit T112 cells indirectly by increasing the release of CCL22—a CCR4 ligand and potent T112 cell chemoattractant—from airway epithelial cells [87]. This indirect effect may be reinforced by the up-regulation of DP1 on airway epithelial cells in the aftermath of antigen challenge [84].

**Cytokine amplification**

Following the early lipid mediator-regulated trafficking of T112 cells into the airways, T112 cell recruitment is amplified dramatically, and the number of T112 cells in the airways correlates positively with asthma severity [88]. The amplification of T112 cell recruitment is initiated by IL-4 and IL-13, released by these first T112 cells recruited to the airways. IL-4 and IL-13 are closely related but act independently in the pathogenesis of asthma [89, 90]. Both cytokines, however, vastly amplify the recruitment of T112 cells into the airways by inducing the release of the T112-active chemokines CCL17 and CCL22, as well as the recruitment of eosinophils by inducing the release of the eosinophil-active chemokines CCL11 and CCL24 from resident lung cells [91–93]. Moreover, IL-13, in particular, acts as an effector molecule, enhancing mucus hypersecretion and airway hyper-responsiveness [90]. In addition to IL-4 and IL-13, T112 cells in the lung release IL-5, which is pivotal for the exacerbation of asthma [90]. In addition to IL-4 and IL-13, T112 cells in the lung release IL-5, which is pivotal for the exacerbation of asthma [90].

**Chemokine recruitment**

“TH2-type” chemokines, induced by IL-4 and IL-13, serve to amplify the recruitment of T112 cells and initiate eosinophil recruitment into the lung. This induction occurs in a STAT6-dependent manner [92]. Consistently, STAT6−/− mice have significantly lower production of the T112-active chemokines CCL17 and CCL22 and the eosinophil-active chemokines CCL11 and CCL24 from resident lung cells [91–93]. Moreover, IL-13, in particular, acts as an effector molecule, enhancing mucus hypersecretion and airway hyper-responsiveness [90]. In addition to IL-4 and IL-13, T112 cells in the lung release IL-5, which is pivotal for the recruitment of eosinophils into the airways [94].

**LIPID-CYTOKINE-CHEMOKINE CASCADE IN INFLAMMATORY ARTHRITIS**

**Lipid initiation**

The existence and importance of chemoattractant cascades initiated by lipid mediators are also exemplified by the recruit-
but not murine TH2 cells. (Middle panel) Recruited TH2 cells release CCL24 (CCR3 ligands), which recruit TH2 cells and eosinophils, re-release CCL17 and CCL22 (CCR4 ligands), as well as CCL11 and resident lung cells, particularly mDCs, but also lung epithelial cells.

Figure 2. Lipid-cytokine-chemokine cascades driving allergic asthma. In allergic asthma, T\(_{H2}\) cells and eosinophils are recruited into the airway lumen by a lipid-cytokine-chemokine cascade consisting of LTB\(_4\)/T\(_{H2}\) cytokines-CCR3/CCR4 ligands. (Left panel) The cascade is initiated after exposure to allergen in the lung with the activation of mast cells by FcεRI crossing, inducing the release of LTB\(_4\) and PGD\(_2\), which recruit the first T\(_{H2}\) cells into the lung interstitium by activating BLT1 and DP2 on T\(_{H2}\) cells, respectively. Of note, DP2 expressed on human but not murine T\(_{H2}\) cells. (Middle panel) Recruited T\(_{H2}\) cells release their signature cytokines IL-4, IL-13, and IL-5. These cytokines activate resident lung cells, particularly mDCs, but also lung epithelial cells and endothelial cells. (Right panel) The aforementioned cells, in turn, release CCL17 and CCL22 (CCR4 ligands), as well as CCL11 and CCL24 (CCR3 ligands), which recruit T\(_{H2}\) cells and eosinophils, respectively, into the lung. Thus, the recruitment of immune cells into the lung is vastly amplified, and allergic asthma is fully established.

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Inflammatory arthritis in the K/BxN model also absolutely depends on IL-1R signaling. Accordingly, mice deficient in IL-1α/β are fully protected from arthritis [117, 118]. Mast cells, platelets, and neutrophils have been suggested to contribute to the production of IL-1α/β in the generation of inflammatory arthritis [118, 119]. We have shown recently that IL-1R signaling is required downstream of BLT1 signaling and that IL-1β derived from neutrophils, may be the major source of IL-1β in this model. The requirement for BLT1 signaling could be overcome by injecting exogenous IL-1β into Libhr1\(^{-/-}\) mice. Additionally, adoptive transfer of IL-1α/β-deficient neutrophils into Libhr1\(^{-/-}\) mice failed to restore arthritis, suggesting that after LTB\(_4\)-BLT1-mediated recruitment into the joint, neutrophils must release IL-1β to induce arthritis. We found that neutrophils isolated from the arthritic joints of K/BxN mice expressed elevated levels of IL-1β mRNA and constitutively released IL-1β protein. The mechanism of IL-1β induction in neutrophils in the joint in vivo, however, remains unknown. In vitro data did not support a direct induction of IL-1β by LTB\(_4\) but instead, hinted at a potential involvement of immune complexes in this process. Further investigations will have to clarify the mechanism of IL-1β induction in vivo. IL-1β levels are also elevated in the arthritic joints of RA patients, and IL-1β expression is also particularly increased in neutrophils infiltrating the synovial fluid, suggesting a role for neutrophil-derived IL-1β in the pathogenesis of RA [120–122].

Chemokine recruitment

CCR1 and CXCR2 ligands are active upon murine neutrophils and contribute to the recruitment of neutrophils into the
joint. Accordingly, a number of CCR1 and CXCR2 ligands are expressed in the synovial tissue of arthritic joints at the mRNA and protein level after K/BxN serum transfer. We found that CCR1-CXCR2 double-deficient mice are completely resistant to arthritis, demonstrating that like LTB₄ and IL-1β, neutrophil-active chemokines are absolutely required for arthritis in this model. Our studies revealed that production of CCR1 and CXCR2 ligands from synovial tissue cells is induced by IL-1β in vivo [10]. Thus, restoration of arthritis in Ltb₄/Cxcr2⁻/⁻ mice by exogenous IL-1β or by adoptive transfer of WT neutrophils resulted in increased mRNA levels of CCL4, CXCL1, and CXCL2 in the synovial tissue of arthritic joints. Inflamed synovial tissue is composed of diverse cell types, including FLS, endothelial cells, and macrophages. These cell types produce different neutrophil-active chemokines following stimulation with IL-1β in vitro, with FLS producing the highest levels in general. FLS produced mainly CXCL5 and lower levels of CXCL1, whereas endothelial cells produced mainly CXCL1 and lower levels of CCL5, CXCL2, and CXCL5. In contrast, macrophages produced mainly CCL9 in response to IL-1β stimulation. These results demonstrate that different cell types in synovial tissue contribute differently to the chemokine milieu in the joint. Intriguingly, neutrophils themselves contribute to the levels of neutrophil-active chemokines in the arthritic joint, producing mainly CXCL2 and lower levels of CCL3, CCL4, CXCL1, and CXCL5.

Although CCR1 and CXCR2 ligands are expressed in the arthritic joint, they did not act in a redundant manner in the generation of arthritis but instead, acted nonredundantly in a sequential manner, with CCR1 ligands acting prior to CXCR2 ligands. Ccr1⁻/⁻ mice developed delayed arthritis compared with WT mice. However, by Day 18, arthritis in these mice became similar with WT mice. Thus, CCR1 ligands predominately participate in neutrophil recruitment in early stages of arthritis and accelerate the inflammatory process but do not appear to be necessary for sustaining arthritis. In contrast, initiation of arthritis in Cxcr2⁻/⁻ mice was similar to WT mice; however, arthritis faltered after 1 week in Cxcr2⁻/⁻ mice and did not reach the peak levels of arthritis seen in WT mice, suggesting that CXCR2 signaling is only required in later stages of arthritis to maximize the recruitment of neutrophils and extend the inflammatory process for longer periods of time. The differential role for CCR1 and CXCR2 in arthritis was also reflected by the time course of the expression of their ligands in the arthritic joint. Thus, although CCR1 and CXCR2 ligands were detectable in joints as early as Day 1 after serum transfer, expression levels of CCR1 ligands peaked on Day 7, whereas levels of CXCR2 ligands continued to soar. This suggests that the different expression patterns of CCR1 and CXCR2 ligands in the course of arthritis may be mainly responsible for the nonredundant roles of CCR1 and CXCR2.

In summary, the studies about inflammatory arthritis reviewed here identify a lipid-cytokine-chemokine cascade, consisting of LTB₄, IL-1β–CCR1/CXCR2 ligands, which drives the generation of arthritis (Fig. 3). Thereby, early LTB₄ production recruits neutrophils into the joint, which in turn, releases IL-1β in the joint to induce the production of neutrophil-active chemokines in the joint. These chemokines—first, CCR1 ligands and later, CXCR2 ligands—amplify the recruitment of neutrophils into the joint. For the generation of full-blown arthritis in this model, this cascade must run its course; otherwise, the inflammatory process is blunted. If such chemok attractant cascades are similarly required for human arthritis, this feature should render arthritis highly vulnerable to therapeutic intervention. The studies discussed here demonstrate neutrophils as pivotal orchestrators of this inflammatory process by virtue of the release of diverse mediators, specifically LTB₄, IL-1β, and CXCL2. This highlights the underappreciated plasticity of this cell type and exemplifies that they are more than mere final effector cells.

CONCLUSIONS

Although in vitro data had suggested previously that chemok attractants act redundantly in the recruitment of immune cells to inflammatory sites, more recent data from in vivo models suggest a nonredundant, cooperative mode of action of chemok attractants in the recruitment of immune cells and this way, in the pathogenesis of inflammatory diseases. Thereby, chemok attractants appear to work in cascades, with one chemok attractant ensuing the release of the next-wave chemok attractants, and this way inducing the recruitment of the next wave of im-
mune cells. As a principle, these cascades of chemoattractants start with the release of lipid mediators, often LTB₄. The reason for this may be the rapid production of lipid mediators after stimulation in comparison with chemokines and cytokines, rendering lipid mediators ideal as initiators of chemoattractant cascades. Remarkably, the principle of lipid mediator-cytokine-chemokine cascade is not limited to a single type of immune response but may be generalized to different pathological processes involving the recruitment of immune cells. As we have discussed, these cascades have been described in such diverse processes as a neutrophil-driven joint inflammation in immune complex-mediated arthritis and TH₂ cell-driven eosinophilic pulmonary inflammation. It will be of interest to determine just how generalizable this process is to immune cell recruitment.

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