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Chemokines in host–parasite interactions in leishmaniasis

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Crucial to the defense against leishmaniasis is the ability of the host to mount a cell-mediated immune response capable of controlling and/or eliminating the parasite. Cell recruitment to the site of infection is essential to the development of the host cellular immune response. The process is controlled by chemokines, which are chemotactic cytokines produced by leukocytes and tissue cells.

Leishmaniasis: a worldwide problem

Leishmania are protozoan parasites that cause a wide spectrum of clinical manifestations. In the skin, these range from localized cutaneous (CL) and mucocutaneous (MCL) leishmaniasis to diffuse cutaneous leishmaniasis (DCL), whereas in the viscera they range from subclinical to potentially fatal disease [1]. The most severe forms are associated either with high parasite numbers and the absence of an effective T helper cell type 1 (Th1) immune response, as seen in patients with visceral leishmaniasis (VL), or with a high inflammatory response with few parasites but exhibiting tissue damage, as seen in MCL [1]. Worldwide, 12 million people are infected with this parasite, and more than 400 000 new cases are reported annually (http://www.who.int/leishmaniasis/burden/en/). Parasites that cause New World CL are grouped under the *Leishmania braziliensis* and *Leishmania mexicana* complexes, whereas those that cause VL are grouped under the *Leishmania donovani* complex [1]. The etiological agents of Old World CL are represented by *Leishmania tropica*, *Leishmania aethiopica* and *Leishmania major*.

Leishmania parasites are obligate intracellular pathogens that preferentially invade macrophages or dendritic cells (DCs) for replication. Early events in host–parasite interactions are likely to influence the future course of the disease. Following infection with *Leishmania* in the skin, a local inflammatory process is initiated; this involves the accumulation of leukocytes at the site of parasite delivery [2]. The composition of the cell populations recruited in this early phase of the infection seems to be essential for defining the outcome of the disease. During this process, members of the chemokine family have a fundamental role in attracting specific subsets of leukocytes to the site of infection and then stimulating them [3] (Box 1). The potential roles of chemokines in *Leishmania* infection include host defense functions such as leukocyte recruitment, participation in cell-mediated immunity, cell activation and antileishmanial activity.

Cytokine–chemokine networks

Cytokines have long been recognized as key elements in the host response against *Leishmania*. Macrophages, which harbor *Leishmania* preferentially, produce interleukin (IL)-1β, tumor necrosis factor α (TNF-α) and IL-12, all products implicated in the inflammatory response. Th1 cells produce interferon (IFN)-γ, and Th2 cells produce IL-4. Other cells are also implicated in cytokine production, with DCs producing IL-12 and natural killer (NK) cells IFN-γ [4,5].

Cytokines are directly involved with chemokine production and can also precede the expression of some chemokines, which, in turn, induce the production of additional inflammatory mediators. Cytokines exert a secondary effect on leukocyte recruitment by inducing the expression of several chemokine genes [6]. TNF-α and IL-1β released from activated neutrophils (PMNs) and macrophages have been implicated in chemokine synthesis in several cell types, including PMNs, fibroblasts, and endothelial and epithelial cells [7]. In leishmaniasis, cytokines seem to synergize with leishmanial elements to regulate chemokine production. TNF-α and IL-1β, together with macrophage inflammatory protein (MIP) 1α (also known as CCL3), were reported to regulate Langerhans cell-mediated transport of *Leishmania* from the infected skin to regional lymph nodes (LNs) in murine CL [8]. IL-12 is required for the induction of Th1-related chemokines such as XCL1 (also known as lymphotactin), IFN-inducible protein 10 (also known as CXCL10 or IP-10; hereafter referred to as CXCL10) and monocyte chemotactic protein 1 (also known as CCL2 or MCP-1; hereafter referred to as CCL2) in LNs of resistant *L. major*-infected mice [9].

Interestingly, Th1- and Th2-derived cytokines can have antagonistic effects on chemokines. For example, some

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Box 1. Chemokines and chemokine receptors

Chemokines are a superfamily of low molecular weight (6–17 kDa) cytokines that recruits distinct subsets of leukocytes and then activates them through increased adhesion, degranulation and the respiratory burst [3]. To date, >44 different chemokines have been described and there are 21 known chemokine receptors.

Most chemokines are secreted proteins of 67–127 amino acids. Their production is stimulated by a variety of agents, including lipopolysaccharides, mitogens, proinflammatory cytokines and several pathogens [7]. The two major structural subfamilies are distinguished by the arrangement of the two amino-terminal cysteine residues, which are either separated by a single amino acid (CXC) or are in adjacent (CC) positions. C chemokines (which lack two out or four canonical cysteines) and CX3C chemokines (with three intervening amino acids between the first two cysteines) are minor structural subfamilies [29].

Chemokine actions are mediated via specific cell-surface receptors, which are members of the seven-transmembrane domain, G-protein-coupled receptor family. The chemokine receptors are named CXC, CC, XC and CX3C, followed by R and a number (according to their ligands: CCR1–10, CXCR1–6, XCR1–2 and CX3CR1). The chemokine–receptor interaction is characterized by considerable promiscuity: each receptor interacts with several chemokines, and each chemokine binds to several receptors [63]. Although the systematic nomenclature has generally been adopted for the receptors, chemokines are still mostly designated by their traditional names, and recently a new nomenclature was adopted [64].

The actions of chemokines are specific to particular cellular groups: members of the CXC class act mainly on PMNs, whereas members of the CC class act on a larger group of cells, including monocytes, basophils, eosinophils, and lymphocytes, but not PMNs. Lymphotactin, the only C chemokine, acts solely on specific subgroups of B and T lymphocytes. Fractalkine, a CX3C-type cytokine, has been reported to attract monocytes, PMNs and T lymphocytes [29].

Previously, chemokines were grouped into the subfamilies termed 'inflammatory' and 'homeostatic' chemokines. However, several chemokines have been described recently as 'dual-function' chemokines. Inflammatory chemokines have broad target-cell selectivity and act on cells of the innate, as well as the adaptive, immune system. Homeostatic chemokines navigate leukocytes during hematopoiesis. Dual-function chemokines participate in immune defense functions and also target non-effector leukocytes [7].

chemokines, such as monokine induced by IFN-γ (also known as CXCL9 or MIG; hereafter referred to as CXCL9) and CXCL10 are more selectively induced by IFN-γ [10]. The Th2-related cytokines IL-4 and IL-13 induce macrophage-derived chemokine (also known as CCL22 or MDC) and CCL6 (also known as C10) production in macrophages, and this production is inhibited by IFN-γ [11,12].

During infection, cytokines can also act synergistically with chemokines. IFN-γ acts with CCL2 to eliminate *L. major* from infected macrophages that have been stimulated by CCL2, whereas IL-4 antagonizes the production of this chemokine by *Leishmania*-infected macrophages [13]. Cytokine regulation of chemokines also appears to be cell specific, as illustrated by the observation that IL-4 and IL-13 strongly induce the production of CCL2 in endothelial cells but inhibit production in epithelial cells [14].

Therefore, there is interplay between cytokines and chemokines. Chemokines are implicated in cell migration and/or activation of both resident and migratory cells, and such cells, in turn, produce cytokines that influence chemokine expression.

The potential roles of chemokines in *Leishmania* infection

Leukocyte recruitment (innate immunity)

Chemokines have different roles in *Leishmania* infection; the most obvious is the recruitment of immune cells to the site of parasite delivery but they also have roles in adaptive immunity and in macrophage activation and parasite killing. The immune response is initiated at the site of pathogen entry by sentinel cells, including DCs, macrophages and γδ T lymphocytes. Such cells are well equipped with Toll-like receptors (TLRs) [15] and phagocytic receptors [16], enabling the detection of pathogen-associated molecular patterns [17] and uptake of pathogens and opsonized particles. Sentinel cells also express various receptors for cytokines and, together with tissue cells, produce numerous chemokines initiating a cascade of innate responses [18]. *L. major*-infected mice induce overall upregulation of CCL5 [also known as regulated on activation normal T cell expressed and secreted (RANTES)], MIP-1α, CXCL10 and CCL2 in the footpads and LNs, whereas these chemokines are constitutive in the spleens of TLR4-competent and -deficient mice. However, the expression patterns are not affected directly by the presence or absence of TLR4 [19].

Infection with *Leishmania* begins when an infected female sand fly takes a blood meal from a human host (Figure 1). The sand fly injects the mammalian host with *Leishmania* within its saliva. Sand fly saliva contains well-characterized molecules that have several activities, including vasodilation, inhibition of coagulation and immunomodulatory effects [20]. It also contains uncharacterized molecules that attract PMNs as well as macrophages [21,22]. The parasite itself also produces a chemoattractant protein called *Leishmania* chemotactic factor, which can attract PMNs [23]. It has recently been shown that, two hours after saliva injection, an intense and diffuse inflammatory infiltrate comprising PMNs, eosinophils and macrophages is induced only in mice pre-exposed to saliva [24].

PMNs are the first cells to arrive at the site of *Leishmania* infection [2]. In humans, PMNs containing *Leishmania* start secreting chemokines such as IL-8 (also known as CXCL8) [25] that are essential in attracting more PMNs to the site of infection. Upon experimental infection with *L. major*, MIP-2 and keratinocyte-derived cytokine (KC; also known as CXCL1), the functional murine homologs of human IL-8 [26], are rapidly produced in the skin [2]. *In vitro* studies have also shown that *L. major* promastigotes induce rapid and transient expression of KC by murine macrophages [27] and of IL-8 by human macrophages [28]. All of these chemokines are chemoattractants for PMNs [29]. PMNs can function as phagocytic cells,

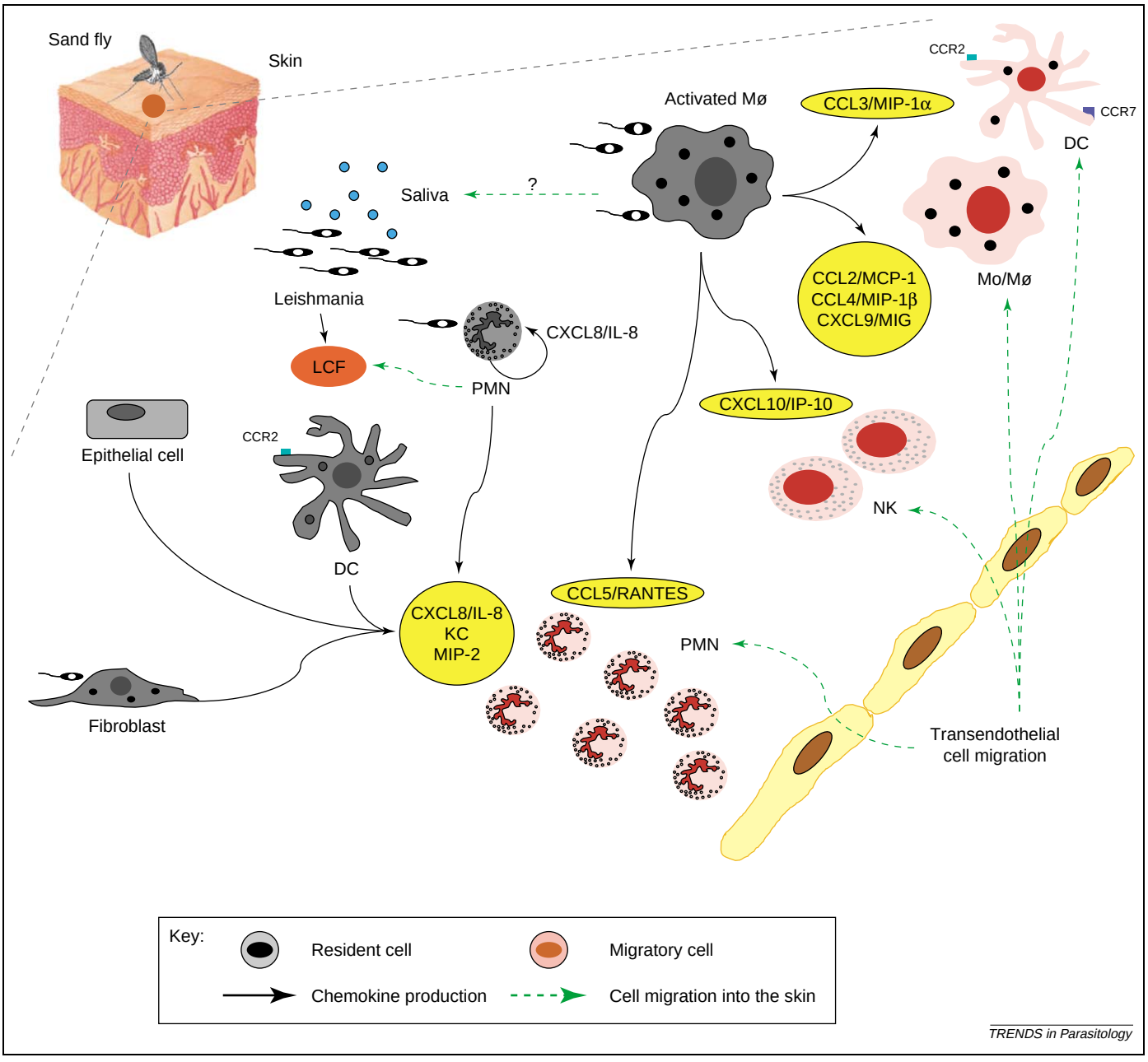

Figure 1. The initial host chemokine interactions in cutaneous leishmaniasis and its effects on the innate immune response. After being inoculated to the host skin by sand fly mouthparts, *Leishmania* promastigotes, together with insect salivary components, induce IL-8, MIP-2 and KC production by sentinel cells (gray). PMNs are attracted early to the site of infection, ingest parasites and produce MIP-1β and IL-8, which, in turn, recruit macrophages (MØ) and more granulocytes, respectively. Recruited macrophages are activated, phagocytose *Leishmania* and release CCL2, which attracts $CCR2^{+}$ cells such as NK cells, DCs and more macrophages. Infected macrophages also secrete other chemoattractants for monocytes (Mo): MIP-1α, MIP-1β and CCL5.

taking up and killing *Leishmania* [30], and they have been implicated in early parasite control.

The role of PMNs in the context of the early response to *Leishmania* has undergone a major change in the past ten years. Early influx of PMNs has been demonstrated to be beneficial for *Leishmania* survival in infected tissue [31]. Moreover, *Leishmania* extends the lifespan of PMNs [32] and can survive intracellularly in these cells for hours or days after infection [31]. After being ingested by PMNs, *Leishmania* induce the release of MIP-1β, recruiting macrophages to the site of infection [31]. Infected PMNs taken up by macrophages do not activate macrophage microbicidal function [31,33]. After ingesting apoptotic PMNs, macrophages undergo inhibition of their proinflammatory cytokine production, through mechanisms involving transforming growth factor-β, prostaglandin E_2 and platelet-activating factor [34,35]. These events contribute to a 'silent' entry of *Leishmania* into macrophages, its main host cell type [36] (Figure 1).

Macrophages are the second wave of cells that enter the site of *Leishmania* infection (Figure 1). They have multiple functions; they serve as host cells for parasite replication, as antigen-presenting cells and as a source of cytokines modulating the T cell-mediated immune response. Moreover, after appropriate activation by Th1

cells, they serve as effector cells for intracellular parasite killing. Monocytes are attracted in the early stages of infection by products of sand fly saliva [21,22] and, two to three days later, by chemokines such as MIP-1β [31]. *Leishmania* can also induce other monocyte-attractant chemokines. Accordingly, *L. major* promastigotes induce rapid and transient expression of JE, a protein inducible by platelet-derived growth factor, in murine macrophages [27] and of its homolog CCL2 in human macrophages [28]. Besides attracting monocytes and macrophages, CCL2 can attract other cells such as NK cells and DCs that are positive for the chemokine receptor CCR2 [13,37]. In human leishmaniasis, CCL2 and MIP-1α seem to be responsible for macrophage activation in the skin lesions. Biopsy samples from patients with *L. mexicana* localized CL exhibited high CCL2 expression and moderate levels of MIP-1α; by contrast, low levels of CCL2 and high levels of MIP-1α were present in the nonhealing DCL lesions [38]. The authors [38] suggest that macrophages stimulated by the synergistic action of CCL2 and IFN-γ kill parasites in localized CL [15], whereas the presence of IL-4 in DCL lesions might suppress CCL2 expression and progression of disease.

NK cells come to the site of infection as early as 24 hours after *Leishmania* infection [2] (Figures 1 and 2). *L. major* infection leads to migration of NK cells, both to the infected skin and into the draining LNs [39]. NK cells are also detectable very early in the lesions of *L. braziliensis*-infected mice [40]. The migration of NK cells correlates with the expression of the NK cell-activating chemokine CXCL10 in resistant

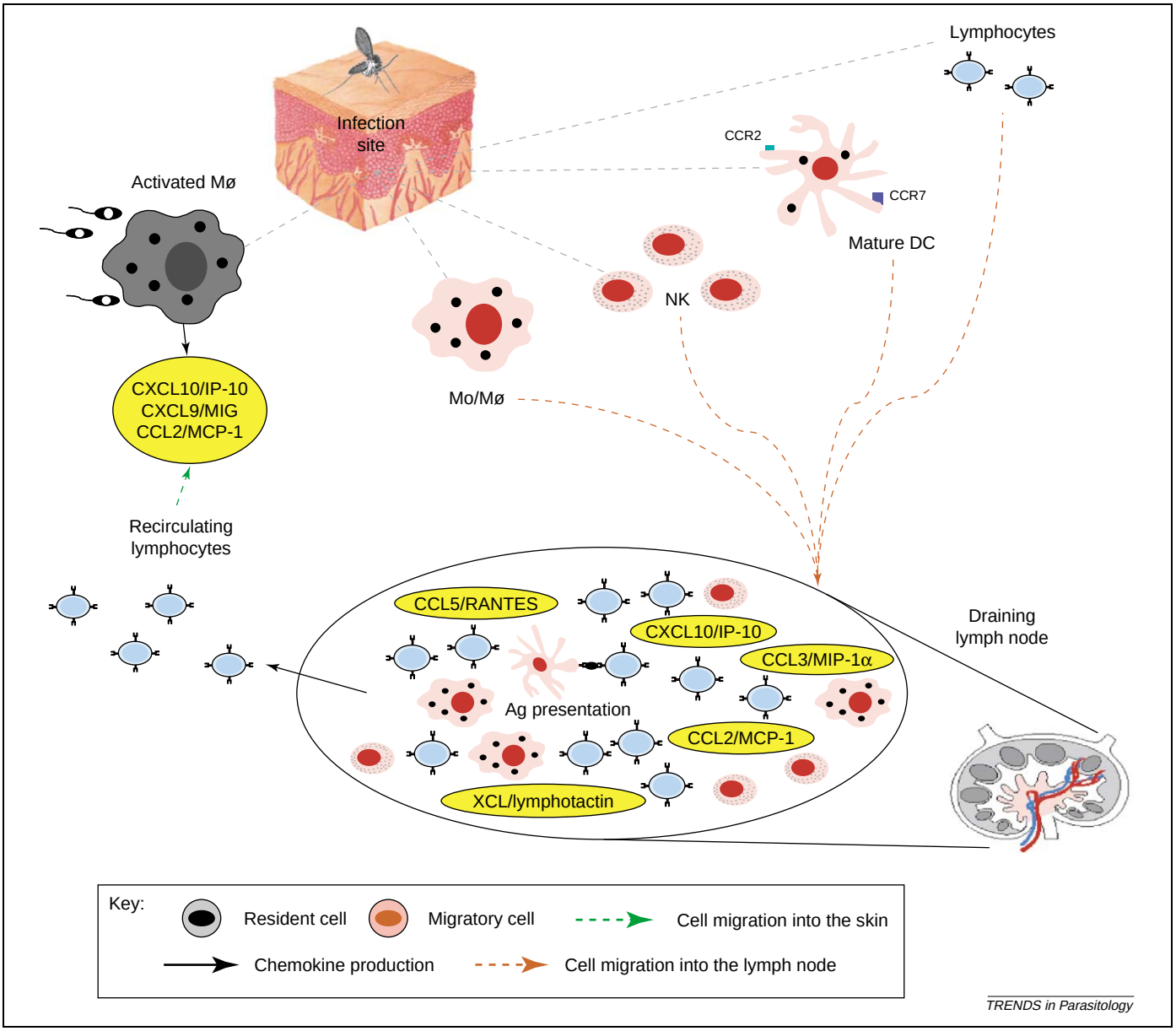

Figure 2. Host innate and adaptive immune response interactions in cutaneous leishmaniasis. Mature DCs, NK cells, macrophages and lymphocytes migrate to the draining lymph nodes, which produce CCL2, CXCL10, CCL5, MIP-1α and XCL1. DCs present antigen to naïve T cells and induce antigen-specific clonal expansion. Therefore, mature Th1 lymphocytes migrate back to the infection site, attracted by CXCL10, CXCL9 and probably CCL2, thereby orchestrating the host delayed type hypersensitivity response against *Leishmania*.

mice [41]. Treatment of susceptible BALB/c mice with recombinant CXCL10 results in significantly increased NK cell cytotoxic activity in the draining LN [2,41]. NK cells are known to produce IFN-γ, and its early activity might influence the kinetics of the Th1 response. Moreover, NK cells have been shown to be important, although not essential, for overall resistance to *L. major* infection [42]. Immune-deficient T cell-reconstituted mice, which selectively lack NK cells, have efficient IL-12-dependent IFN-γ production by $CD4^+$T-cells and heal their lesions [43].

Cell-mediated immunity (adaptive immunity)

Cutaneous leishmaniasis

Skin DCs, which are potent antigen-presenting cells, have a decisive role as a bridge from innate to adaptive immune responses by priming naïve T cells (Figure 2). DCs take up *Leishmania* parasites, acquire a mature phenotype by upregulation of class I and II major histocompatibility complex surface antigens, increase their expression of co-stimulatory molecules (CD40, CD54, CD80 and CD86), release IL-12 p40 and transport the parasites from the infected skin to the draining LNs for presentation to antigen-specific T cells [44]. In *Leishmania*-infected mice, the ability of DCs to transport the parasites to the draining LNs seems to rely on the expression of CCR2 and CCR7. In CCR2-deficient mice, which are susceptible to *L. major* infection, DC migration to the draining LN and spleen was found to be markedly impaired, especially for the CD8α+ Th1-inducing DC subset, and these mice had a dominant Th2 phenotype [45]. CCR7 is also required for the migration of mature DCs from tissues to T cell areas of draining LNs [46]. *L. donovani*-induced down-regulation of CCR7 impairs DC migration, contributing to disease progression [47].

Indeed, mice lacking CCL2, a major CCR2 ligand, have impaired Th2 responses but secrete normal amounts of IFN-γ and are resistant to *L. major* infection [48]. This discrepancy might not be surprising, given the fact that CCR2 has at least two additional high-affinity ligands in the mouse [CCL7 (also known as MCP-3) and CCL12 (also known as MCP-5)], which, in the appropriate context, induce Th1 polarization in CCL2-deficient mice. Mature Th1 lymphocytes migrate back to the infection site, attracted by CXCL10, CXCL9 and probably CCL2, as seen in the lesions of patients with localized CL [38]. In summary, it is clear that the CCR2–CCL2 axis participates in innate immunity to *Leishmania* infection, such as in cell recruitment, but its participation in adaptive immunity through control of Th1 versus Th2 balance still remains controversial.

Other chemokine receptors have also been studied. CCR1-deficient C57BL/6 mice infected with *L. major* showed that CCR1 (which is preferentially expressed on $CD4^+$ Th1 cells) upregulates the production of Th2-type cytokines such as IL-4 and IL-10 in the early course of disease [49]. However, CCR1 is not essential for T cell and macrophage trafficking, either to the site of infection or to the LNs following *L. major* infection [49]. By contrast, CXCR3 has a crucial role in the host defense against CL caused by *L. major*. $CXCR3^{-/-}$ mice mount an efficient Th1 response, evident by IL-12 production, but fail to control the parasite replication that is associated with lower IFN-γ production in the lesion compared with $CXCR3^{+/+}$ mice [50].

Visceral leishmaniasis

In VL, some chemokines and chemokine receptors have a role in the development of the Th1 response because their deletion influences IFN-γ production by T cells. Following the ligation of T cell receptors in *L. donovani* infection, IFN-γ is produced, inducing macrophage activation and the killing of parasites. *L. donovani*-infected mice lacking CCR5 or MIP-1α (a ligand for CCR5) demonstrate a low antigen-specific IFN-γ response during the early phases of infection [51]. This defective response is transient because it is restored during chronic infection and it correlates with enhanced control of parasite replication. In this system, however, CCR5 and MIP-1α are not essential for the containment of murine infection.

Chemokines also seem to be implicated in T cell amplification of the inflammatory response, an important step for protective host defense in VL. *L. donovani*-infected mice undergo a rapid hepatic accumulation of MIP-1α, CCL2 and CXCL10 after infection [52]. However, only CXCL10 expression, amplified by T cells, remains high during the late phase, and this is essential to enable liver granuloma formation and the inflammatory response. Monocytic cells attracted by MIP-1α and CCL2, and following IFN-γ stimulation, could be the source of Th1-mobilizing chemokines such as CXCL10 [10]. Unlike liver cells, spleen cells from *Leishmania infantum*-infected mice produce both Th1- and Th2-type cytokines, with the Th2-type response being dominant. This is compatible with the sustained expression of CCL2 rather than CXCL10, thereby showing that there is an influx of macrophages rather than T cells in the spleen [53] (Figure 3). This explains, in part, why the liver usually controls the infection, whereas parasites persist in the spleen [54].

Macrophage activation and parasite killing

In leishmaniasis, some chemokines such as CCL2 can activate macrophages that participate in reducing parasite numbers [15,38]; these chemokines also induce antileishmanial activity in both *L. donovani*-infected and *L. major*-infected human macrophages [13,38]. CCL2 and MIP-1α can induce leishmanicidal ability *in vitro* in *L. infantum*-infected human macrophages and can control the intracellular growth and multiplication of *L. donovani* via a nitric oxide-mediated regulatory mechanism [55].

Chemokines and parasite virulence

In addition to other factors, the virulence of *Leishmania* seems to be linked to the early modulation of chemokine expression in the host (Table 1). Some *Leishmania* strains might evade host immune responses by preventing the early production of cytokines, chemokines and chemokine receptors, and thus impairing antigen-specific Th1 cell development. Mice infected with *Leishmania amazonensis* had delayed and reduced MIP-1α, MIP-1β, CCL5, MIP-2,

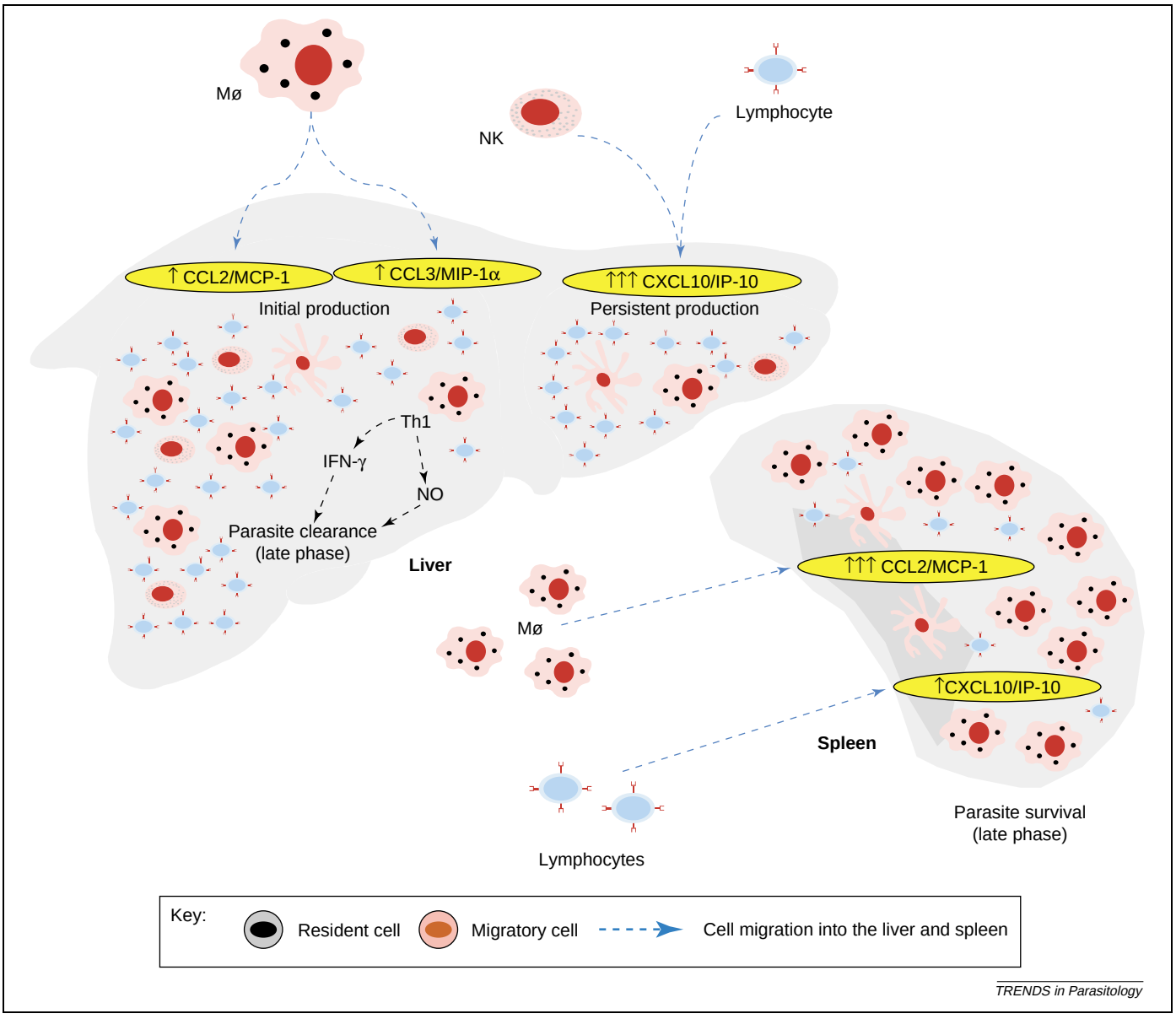

Figure 3. Role of chemokines in visceral leishmaniasis. After *Leishmania* infection, a rapid hepatic accumulation of CCL2, MIP-1α and CXCL10 is seen. The CXCL10 expression remains high over time postinfection and this attracts more Th1 cells than macrophages to liver, resulting in hepatic parasite control. By contrast, in the spleen, a consistent expression of CCL2 rather than CXCL10 is seen, leading to an influx of macrophages rather than T cells, with a dominance of Th2 cytokines and sustained parasite persistence.

CCR1, CCR2 and CCR5 expression in the early stages of infection, compared with *L. major*-infected mice. These alterations were accompanied by reduced responsiveness of T cells [56]. Recently, it has been shown that lesions from BALB/c mice caused by a more pathogenic *L. braziliensis* strain have a higher expression of CCL2, MIP-1α, KC, CCL11 (also known as eotaxin), XCL1 and their respective receptors when compared with lesions caused by a less pathogenic *L. braziliensis* strain [57]. This higher expression of chemokines correlates with recruitment of more leukocytes to the lesions, resulting in the increased inflammation observed in pathogenic *L. braziliensis*-infected mice. However, studies performed with murine macrophages show that the induction of chemokines upon infection with *Leishmania* is dependent on parasite virulence; the magnitude of CCL2 and KC expression is higher with avirulent than with virulent strains of *L. major* [27]. The differences in CCL2 expression reported here might be related to the *Leishmania* strain used (*L. major* versus *L. braziliensis*), the kinetics of infection (early versus late infection) and the experimental system used (*in vitro* versus *in vivo*).

The parasite lipophosphoglycan (LPG), a major surface molecule that binds to macrophage surface receptors, seems to be involved in modulating the signal for chemokine induction. *L. donovani* LPG alters the migration of monocytes by decreasing the expression of adhesion molecules and inhibiting the induction and release of CCL2 [58]. Indeed, the expression of the genes encoding CCL5, MIP-1α, MIP-1β, CXCL10 and CCL2 is more strongly upregulated in the air pouch lining of viscerotropic *L. donovani*-infected animals than in that of dermotropic *L. major*-infected animals [59], suggesting that leukocyte transendothelial migration could be

Table 1. Chemokines and chemokine receptors in *Leishmania* infection

Species	Modulation	Effect	Refs
Cutaneous leishmaniasis			
Initial phase			
L. major	↑KC, IL-8, MIP-2	Attract PMNs (*in vivo* and *in vitro*)	[2,25,27,28]
L. major	↑MIP-1α, MIP-1β CCL5	Attract monocytes and macrophages (*in vivo* and *in vitro*)	[59,31]
L. major	↑CXCL10	Activate and recruit NK cells to lesion and lymph nodes (*in vivo*)	[2,39,41,57]
L. braziliensis			
L. major	↑CCL2	Recruits macrophages, NK cells and DCs (*in vitro*)	[27,28,37]
L. major	↑CCR2, CCR7	DC migration to lymph nodes and co-localization in T cell areas (*in vivo*)	[45,46]
L. braziliensis	↑CCL2, MIP-1α, KC, XCL1	Increase inflammation in the lesion (*in vivo*)	[57]
Late phase			
L. major	↑CCR2–CCL2	Control of Th1 versus Th2 polarization (?) (*in vivo*)	[45,48]
L. major	↑CCR1	Upregulation of Th2 type cytokines (*in vivo*)	[49]
Leishmania amazonensis	↓MIP-2, MIP-1α, MIP-1β, CCL5, CCR1, CCR2 and CCR5	Impairment of antigen-specific Th1 cell response (*in vivo*)	[56]
Visceral leishmaniasis			
Initial phase			
L. donovani	↑MIP-1α, CCL2	Attract monocytic cells as a source for Th1-mobilizing chemokines (*in vivo*)	[52]
L. donovani	↓CCR7	Impaired DC migration to lymph nodes (*in vivo*)	[47]
Late phase			
L. donovani	↑CXCL10	Participates in granuloma formation and attracts lymphocytes (liver) (*in vivo*)	[52]
L. donovani	↑CCR2, CCR5 MIP-1α	Role in generation of IFN-γ by T cells (*in vivo*)	[51]
L. donovani	↑CCL2 ↓CXCL10	influx of macrophages and sustained parasite persistence (spleen) (*in vivo*)	[53]

blocked by *L. donovani* LPG [60]. Infective *Leishmania* promastigotes also express on their surface gp63 protease and an abundant class of small glycolipids termed glycosylinositolphospholipids [61]. Although there is evidence showing the importance of LPG and gp63 in determining the virulence of *Leishmania* promastigotes, the role of these molecules in providing the signal for chemokine induction should be investigated further because amastigotes lose or downregulate the expression of these molecules [61].

Chemokines as targets for therapy in leishmaniasis

Until now, only two studies using chemokine blockade or recombinant chemokine treatment for leishmaniasis have been reported. The administration of recombinant mouse CXCL10 to susceptible BALB/c mice significantly enhanced NK cell cytotoxic activity and resistance against *L. major*, indicating that CXCL10 might contribute to promoting the development of a protective immune response [41]. Treatment with Met-RANTES (a functional antagonist of CCR1 and CCR5) or anti-CCL5 rendered C57BL/6 mice more susceptible to *L. major*, skewing the immune response towards Th2 [62]. Whether chemokines can be exploited therapeutically to limit the extent of inflammation, and whether this would be beneficial in leishmaniasis, is uncertain. The feasibility of these mediators for the treatment of inflammatory diseases will become clearer as a more complete understanding of the biology of chemokines emerges.

Concluding remarks

Studies of leishmaniasis reinforce the notion of redundancy in chemokine action; however, to prove this concept, *in vivo* inhibition of certain chemokines will need to be achieved because subtle *in vivo* regulation might not be detectable in current approaches. Despite this note of caution, some theories concerning of the role of chemokines on the physiopathology of leishmaniasis have been proposed (Box 2). *Leishmania* can invade many types of cells (macrophages, DCs and fibroblasts), and the chemokines thereby released attract immune cells. Whether such incoming cells act as hosts to the parasite, contributing to its virulence, or whether they are major contributors to defense, mediating innate resistance to intracellular pathogens, might be influenced by which chemokines participate in the process. For example, resistance against *Leishmania* might depend on special combinations of recruited cells. Additionally, different chemokines might recruit the same cells but differ in the state of activation they induce in them. Even though much less is known concerning the involvement of chemokines in protozoan diseases compared with viral diseases, it is clear that chemokines are of paramount importance in the pathophysiology of leishmaniasis. It is likely that the concerted and timely actions of several chemokines and chemokine receptors are relevant in this regard. The

Box 2. Future challenges regarding chemokines in *Leishmania* infection

- Explore immunopathogenesis through the use of inhibitors of the chemokine system
- Investigate the role of chemokines in inducing different stages of cell activation in cells which have been recruited early postinfection
- Understand the role of distinct parasite strains or parasite stages (e.g. amastigotes) in altering host chemokine expression
- Search for and test key chemokines which might be useful in vaccination against leishmaniasis
- Identify the target chemokines to block to limit tissue damage in the hyperergic forms of leishmaniasis

development of inhibitors of the chemokine system provides hope that such interventions will unravel novel aspects of pathogenesis and open new therapeutic avenues in leishmaniasis.

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