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Chemokines shape the immune responses to tuberculosis

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Abstract

Mycobacterium tuberculosis (Mtb) is the intracellular pathogen that causes the disease, tuberculosis. Chemokines and chemokine receptors are key regulators in immune cell recruitment to sites of infection and inflammation. This review highlights our recent advances in understanding the role of chemokines and chemokine receptors in cellular recruitment of immune cells to the lung, role in granuloma formation and host defense against *Mtb* infection.

Keywords

Tuberculosis; Chemokines; lung

1. Introduction

Mycobacterium tuberculosis (Mtb) is an intracellular pathogen that is transmitted via the respiratory route and is the causative agent of the disease, tuberculosis (TB). *Mtb* continues to infect approximately one-third of the world's population, with a majority of infected individuals maintaining immune control in the form of a latent infection [1]. However, *Mtb* can persist for the life of the host and reactivation can occur in ~5-10% of latently infected individuals, a majority within 2-3 years after exposure. Upon entry of *Mtb* into the airways of the lung, one of the first lines of cellular defense against the invading bacterium is the alveolar macrophage [2]. However, *Mtb* utilizes several mechanisms to subvert macrophage activation allowing replication within macrophages, while inducing expression of inflammatory cytokines and chemokines in the lung. Simultaneously, dendritic cells (DCs) that encounter *Mtb* in the lung act as primary antigen-presenting cells and migrate to the draining lymph node to drive proliferation and differentiation of effector T cells [3]. In response to inflammatory infection-induced chemokine signals in the lung, effector T cells, B cells and other innate cells such as neutrophils and monocytes accumulate in the lung and form an organized structure called the granuloma. In this context, the production of proinflammatory cytokines such as Interferon gamma (IFN γ) and Tumor Necrosis Factor alpha (TNF α) by effector immune cells is pivotal for control of *Mtb* infection [3]. In addition, granulomas provide an immunological environment where adaptive and immune cells with anti-mycobacterial function interact to mediate *Mtb* control thereby preventing dissemination of the bacterium to the periphery [2]. In contrast, the granuloma has also been

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proposed to serve as a unique niche for persistence of latent *Mtb*, which under conditions of immune suppression allows for *Mtb* reactivation [4]. In this review, we will summarize recent insights into our understanding of chemokines that are induced in the lung in response to *Mtb* infection, innate and adaptive immune cells that express the necessary chemokine receptors and respond to these chemokines to accumulate in the lung and mediate formation of granulomas, bacterial control and protection against *Mtb* infection.

2. Chemokine induction in the lung in response to *Mtb* infection

In 1988, several groups described a new 6500 MW human monocyte-derived neutrophil chemotactic factor and stimulating peptide, which we now know as IL-8 [5-8]. Within the decade following this initial discovery of IL-8, 50 or so ligands and at least 20 receptors have been identified to be involved in leukocyte trafficking giving rise to the group of chemotactic cytokines called “chemokines” (Table 1). Chemokines are now categorized into four subfamilies based on the location of their cysteine residues: C, CC, CXC, CX₃C [9-10] and are important in regulating inflammation, angiogenesis, leukocyte recruitment, and antimicrobial immunity [11-12]. The availability of several animal models of TB [13], such as the well described mouse model, guinea pig model and the NHP model has greatly benefited our overall understanding of chemokines in *Mtb* infection. Despite the fact that granulomas seen in the mouse model of TB are not organized similar to tubercle granulomas seen in *Mtb*-infected humans and NHPs, use of novel chemokine gene deficient mice in the mouse model of TB has been pivotal in documenting the individual as well as overlapping and redundant roles of chemokines and their ligands in *Mtb* infection. In the mouse model of low dose aerosol *Mtb* infection, mRNA for a number of chemokines and their corresponding receptors have been reported to be upregulated in the lung between days 12-21 post infection (Table 2) [14] and this likely correlates with the recruitment of immune cells into the lung (Figure 1). Specifically, the early accumulation of a variety of different innate cells such as neutrophils, NK1.1 cells, $\gamma\delta$ T cells and macrophages (Figure 1a), as well as later accumulation of adaptive effector cells such as CD4⁺ T cells, CD8⁺ T cells and B cells appear to coincide with induction of specific chemokine mRNA in *Mtb*-infected murine lung (Figure 1b). For example, CXCL3 and CXCL5 mRNA is induced early by days 12-15 post *Mtb* infection in the murine lung (Table 2) [14], which may correlate with early accumulation of neutrophils and NK cells within the lung (Figure 1a). The corresponding receptor CXCR2, is known to be expressed on neutrophils [15] and NK cells [16] and it is likely that these ligands act in a redundant manner to recruit CXCR2-expressing immune cells to the lung. Further, CXCL9 mRNA is upregulated by day 12 post infection followed closely by the redundant ligands CXCL10 and CXCL11 mRNA at day 15-21 post infection. This results in the coincident induction of CXCR3 mRNA in the lung at day 21 [14], likely as the responding CD4⁺, NK T cells and CD8⁺ T cells infiltrate the lung (Table 2 and Figure 1). Constitutive expression of “homeostatic” chemokines accounts for the accumulation of naïve T cells, B cells and resident macrophages in secondary lymphoid organs (SLO), as well as for the recruitment of dendritic cells following antigen exposure. Four chemokines are responsible for SLO organization, specifically CCL19, CCL21, CXCL12, and CXCL13. Of these CCL19, CXCL12 and CXCL13 mRNA is upregulated in the lung at D21 during an *Mtb* infection (Table 2)[14]. Similarly, in the non-human primate model of active TB, several different chemokine mRNAs are induced in the lung following infection [17]. These studies together suggest that induction of chemokines in response to inflammatory signals induced by *Mtb* infection may play a role in recruitment of immune cells to the lung to mediate control of *Mtb* infection.

2. Chemokines in innate and adaptive immunity to *Mtb* infection

There are at least 46 chemokines in humans that are known to interact with a number of G-protein linked receptors [11]. The CC-chemokine family binds to the chemokine receptors termed CCR1-11 and the CXC-chemokine family binds to the chemokine receptors CXCR1-7. The role of these receptors and ligands in the establishment of cell-mediated immunity and granuloma formation during *Mtb* infection has just begun to be elucidated.

2.1 CC-Chemokine Receptors

2.1.1 CCR2—The monocyte chemoattractant proteins (MCPs such as CCL 2,7,8,12,13,16) are potent attractants of monocytes, DCs, memory T cells, and natural killer (NK) cells to sites of inflammation [18] [19]. Accordingly, CCL2 (MCP1) mRNA is induced in the *Mtb*-infected murine lung at day 15 and coincides with induction of the receptor, namely CCR2 mRNA at day 21 (Table 2)[14]. In addition, CCL2 is produced by human peripheral blood mononuclear cells (PBMCs) and is expressed at elevated levels in the sera and pleural fluid of patients with active TB [20-21]. Consistent with these observations, human whole blood Th cells, neutrophils and NK cells of pulmonary TB patients express higher levels of the coinciding receptor CCR2 [22]. To mechanistically address the role of CCR2 in mediating cellular recruitment during *Mtb* infection, CCL2 and CCR2 deficient mice were infected with *Mtb* and disease phenotype assessed. Interestingly, CCL2 knock out (KO) mice receiving a high intravenous dose of *Mtb* were not more susceptible than wild-type *Mtb*-infected mice [23]. In contrast, CCL2KO mice infected with a low doses of aerosolized *Mtb* had a small and transient increase in lung bacterial burden early following infection [24]. This increased susceptibility was associated with decreased macrophage influx and reduced accumulation of IFN γ -producing CD4⁺ T cells, suggesting a potential role for CCL2 in both macrophage and T cell recruitment [24]. In reciprocal studies, CCR2 deficient mice were found to be more susceptible to systemic high dose *Mtb* infection and exhibited increased pulmonary cellular composition due to higher accumulation of neutrophils [25-26]. The increased susceptibility seen in the CCR2KO [26] but not CCL2KO mice [23] in the high dose systemic model of *Mtb* infection is likely due to the redundancy in the multiple CCL chemokines that all utilize CCR2 as a receptor. Using bone marrow chimeric mice to determine whether the increased susceptibility of the CCR2KO mice to high doses of systemically delivered *Mtb* was due to impaired trafficking of monocyte/macrophages and dendritic cells or T cells (or both), it was found that expression of CCR2 on macrophages and/or dendritic cells was required to mediate protective immunity against systemic *Mtb* infection [27]. The increase in susceptibility in this high dose systemic *Mtb* infection model in the CCR2KO mice was attributed to a delay in myeloid cell-dependent T cell recruitment to the lung, T cell polarization and subsequent activation of macrophages. Interestingly, CCR2KO mice are not more susceptible to low doses of systemically delivered *Mtb* or to infection with low doses of aerosolized *Mtb* [25]. That CCR2KO mice can control *Mtb* pulmonary infection despite decreased monocyte/macrophage and T cell numbers in the lungs [25], suggests that although CCR2 and CCL chemokines play a role in macrophage recruitment to the lung and subsequent T cell polarization, absence of its expression is likely compensated by expression of other inflammatory chemokines and their corresponding receptors. Therefore, it is interesting that certain CCL2 polymorphisms in human population genetic studies, specifically -2518 allele, is associated with increased risk of developing TB [28].

2.1.2 CCR4—The chemokine receptor CCR4 is widely expressed on cells of the immune system including NK cells, macrophages, dendritic cells, T cells, and basophils [29-30]. Although CCR4 was initially considered to be selectively expressed on T helper type 2 cells (Th2) and T regulatory cells [31-33], more recent evidence suggests that other helper

subsets, such as Th1 cells [34-35] and T helper type 17 (Th17) cells [36-37] can also express this receptor. Further, CCR4 is regarded as a primary receptor expressed on lymphocytes homing to the skin during inflammatory and autoimmune skin diseases [38-39]. However, some recent evidence also suggests that CCR4 participates in migration of Th cells to the lungs [34, 40]. During murine *Mtb* infection, mRNA for one of the CCR4 ligands, CCL22/MDC is upregulated by day 21 post infection, while CCL17/TARC mRNA is induced transiently at earlier time points (Table 2)[14]. Interestingly, using an antigen coated bead model where granulomas were induced in the lungs of *M.bovis* PPD sensitized mice [34, 41], CCR4 and both its responding ligands CCL17 and CCL22 mRNA, were found to be upregulated in the sensitized lung [34]. Furthermore, CCR4 mRNA expression was enriched in the IFN γ -producing CD4⁺ T cells isolated from the sites of granulomas in PPD sensitized mice. Consistent with these data, instillation of CCR4KO mice with PPD coated beads in the lung resulted in impaired granuloma formation and exhibited reduced IFN γ production at the lesion site [34]. In addition, in a model of acute *M.bovis* pulmonary infection, CCR4KO mice exhibited higher susceptibility with increased bacterial burden in the lung early in infection [42]. The increased susceptibility in CCR4KO mice also coincided with formation of smaller granulomas and reduced accumulation of innate NKT cells as well as defects in late stage CD4⁺ T cells that produced the cytokines, IFN γ and IL-17 [42]. Although these studies indicate a potential role of CCR4 in immunity to mycobacterial infections, following infection with low doses of aerosolized *Mtb*, CCR4KO mice are not more susceptible and exhibit comparable bacterial burden in the lungs as wild type *Mtb*-infected mice (*Slight and Khader, unpublished findings*). These studies together suggest that although CCR4 may play a role in recruitment of immune cells in acute models of mycobacterial pulmonary infections, CCR4 expression may be dispensable for overall protective immunity against pulmonary *Mtb* infection.

2.1.3 CCR5—The chemokine receptor 5 (CCR5) is expressed by macrophages, immature dendritic cells, granulocytes and T cells (both CD8⁺ and CD4⁺) [43], specifically so on Th1 cells [44]. Cell migration of CCR5⁺ expressing cells to sites of infection is known to be mediated by a redundant system of ligands: CCL3, CCL4, CCL5, and CCL8 [11]. These chemokines are induced in lungs of *Mtb*-infected NHPs [17] and mice (Table 2) [45-48] and correlates with the accumulation of CCR5⁺ immune cells during *Mtb* infection [46, 49-51]. In the mouse model, the importance of CCR5 during mycobacterial infections has been explored using mice deficient in CCR5 and its corresponding ligand CCL5. CCR5 deficient mice were found to have increased pulmonary lymphocytic and myeloid cell infiltration that coincided with higher induction of IFN γ , NOS2, and IL-12 mRNA and effective control of *Mtb* infection [45]. In addition, although CCR5KO *Mtb*-infected mice exhibited increased lymphocytic infiltrates, they were able to develop normal granulomas as their wild type counterparts [45, 47]. In contrast, when CCL5 deficient mice were tested in the low dose aerosol *Mtb* infection model, they displayed higher bacterial burden in the lung early following infection, and this coincided with a transient delay in lymphocytes and CD11c⁺ myeloid cell infiltration to the lung [46]. These data suggest that CCL5 expression may be required for optimal initiation of T cell priming during *Mtb* infection and control of *Mtb* infection.

CCR5 is a major coreceptor for HIV [52-53] and clinical trials testing CCR5 antagonists have been completed and are efficacious in HIV treatment [54]. However, since *Mtb* is the leading cause of death among HIV infected patients, the effect of CCR5 antagonist treatment regimens on *Mtb* containment raises concerns [43]. An increase in CCR5 expression on CD4⁺ T cells from peripheral blood and BAL from active TB patients has been documented [51]. These studies suggest during *Mtb* infection, CCR5⁺ T cells accumulate in the lung and may provide a preferential site for increased HIV replication, resulting in viraemia and deletion of *Mtb*-specific T cells required for control of *Mtb*

infection [50, 55-56]. In addition, that certain polymorphisms in the *CCL5* promoter are associated with increased susceptibility to TB [57-58] suggest that *CCL5* expression may be important for overall protective immunity against TB.

2.1.4 CCR6—CCR6 is unique receptor in that it thus far has only been reported to bind to one ligand, namely CCL20. CCR6 is expressed on effector/memory T cells, myeloid dendritic cells, and B cells and has been documented for recruitment of these immune cells to gut mucosal lymphoid tissues as well as sites of epithelial inflammation [59]. During *Mtb* infection, CCL20 mRNA is upregulated in murine lung (Table 2) [14] and NHPs [17]. In addition, higher levels of CCL20 were detected in PBMCs, monocyte-derived macrophages (MDMs), and bronchoalveolar lavage fluid (BAL) from TB patients compared with healthy controls [60-61]. Using CCR6 deficient mice in an acute *M. bovis* BCG pulmonary infection model, it was reported that CCR6 expression was not required for DC function or induction of adaptive T cell immunity, but was critical for optimal innate immune mediated mycobacterial clearance. Accordingly, Cd1b-restricted iNKT cell accumulation was reduced in lungs of CCR6 deficient mice and resulted in increased susceptibility to *M. bovis* BCG infection [62]. More recently, Th17 cells have been shown to specifically express CCR6 [37] and human *Mtb*-specific Th cells producing both IFN γ and IL-17 were found to co-express CXCR3 and CCR6 [63]. These studies suggest that CCR6 may be one of the alternate chemokine receptors used by activated T cells to respond to inflammation induced chemokine signals and home to the lung. Since both IL-17 and IFN γ -producing T cells play key roles in immunity to TB [64], future studies addressing the specific and redundant roles of CCR6 in generating effective primary and secondary immune responses to *Mtb* infection will be necessary.

2.1.5 CCR7—Migration of T cells and DCs to the paracortical T-cell zones of secondary lymphoid organs is dependent upon the expression of the homeostatic chemokines CCL19 and CCL21. CCL19 and CCL21 are expressed by resident stromal cells, while CCL21 is expressed on the lymphatic endothelium and high endothelial venules (HEVs) [65]. Naïve and central memory T cells, as well as DCs express CCR7 and respond to this chemokine gradient [66-67] and the expression of these chemokines are required for effective T-cell priming [65, 68-69]. Accordingly, following *Mtb* pulmonary infection, DCs upregulate CCR7 [70-72] and migrate to the draining lymph node [72-73]. Consistent with this role for CCR7 in DC migration, CCR7 deficient mice exhibit impaired DC migration to the mediastinal lymph node, resulting in delayed dispersion of *Mtb* to peripheral organs [74-75], and delayed activation of T cells [75]. Similarly, mice that have a genetic mutation leading to the loss of CCL19 and CCL21 expression (plt mutant mice) have defects in DC migration to the DLN [73] and decreased induction of T cells producing IFN γ in the lymphoid organs [76]. The delayed generation of IFN γ -producing activated T cells in the lymphoid organs correlated with delayed accumulation of IFN γ producing T cells in *Mtb*-infected lungs and resulted in increased susceptibility to *Mtb* infection [76]. These data together support a role for CCL19 and CCL21 expression in the lymphoid organs to mediate optimal DC migration and T cell priming during *Mtb* pulmonary infection. Furthermore, CCL19 (but not CCL21) mRNA is also induced in the lung following *Mtb* infection (Table 2) [14], and plt mutant mice do not form organized granulomas and lack organized lymphoid structure formation [76]. Therefore, it is possible that there is a role for lung CCL19 expression that is independent of its role in DC migration and T cell priming, and this needs to be carefully addressed.

2.2 CXC-chemokine receptors

2.2.1 CXCR1 and CXCR2—Chemokines in the CXC-family can be characterized by the presence or absence of a tripeptide motif ELR (Glu-Leu-Arg) at the amino terminus of the

protein. Those chemokines containing the ELR motif, specifically CXCL1-3 and CXCL5-8 recruit neutrophils through CXCR1 and CXCR2, to sites of inflammation [77]. CXCL8 (also known as Interleukin 8) and CXCL6 respond to both CXCR1 and CXCR2 while the remaining ELR+ chemokines all respond to CXCR2 (Table 1). Although neutrophils are considered primary cells that express CXCR1/2, PBMCs isolated from human TB patients express CXCR2 on neutrophils, NK cells and Th cells [21-22]. In addition, a separate study showed that both monocytes isolated from TB patients and latent TB patients expressed CXCR1 and CXCR2 [78], suggesting CXCR1/CXCR2 expression on immune cells other than neutrophils may play a role during *Mtb* infection. However, human dextran purified neutrophils from TB patients also exhibited increased expression of CXCR2 when compared to neutrophils from normal individuals [79]. CXCR1/CXCR2 ligands are produced by a number of inflammatory cells in mice, NHPs and humans infected with *Mtb* [22, 80-85]. Pulmonary fibroblasts within human tuberculosis granulomas express CXCL8 [82], while in vitro treated monocytes [86], macrophages [87], alveolar epithelial cells [88] and fibroblasts [82] can all produce CXCL8 upon *Mtb* exposure. Neutralization of CXCL8 during Delayed type hypersensitivity (DTH) reaction in rabbits inhibits the associated inflammatory process [89], suggesting that CXCL8 may have a role to play in leukocyte accumulation during TB. Consistent with this, CXCL8 levels in sputum from TB patients closely parallels and precedes mycobacterial clearance in patients undergoing TB therapy, suggesting that CXCL8 may be a useful early surrogate marker in response to TB therapy [90]. Since CXCL8 gene polymorphisms are associated with higher TB susceptibility in some populations [91] but not others [92], a more detailed examination of the role of CXCL8 in TB is warranted. However, absence of CXCL8 homologue in mice makes it difficult to mechanistically address the role of CXCL8 during *Mtb* infection in vivo [11, 93]. Therefore, mice deficient in the receptor CXCR2 have been studied in mycobacterial infection models. Following intraperitoneal infection with *M. avium*, CXCR2 deficiency resulted in decreased neutrophil recruitment to the peritoneal cavity and showed increased bacterial burden in target organs [15]. However, CXCR2 deficient mice that received pulmonary *M. avium* infection did not show decreased neutrophil recruitment or increased susceptibility to mycobacterial infection [15]. Therefore, although it appears that CXCR1/CXCR2 may be important for both cellular recruitment and mycobacterial killing, its exact role in vivo during *Mtb* infection has not been directly addressed.

2.2.2 CXCR3—CXCR3 is an inflammatory chemokine receptor that is upregulated on naïve T cells rapidly following DC: induced T cell activation [94]. T-bet, the Th1 master transcription factor directly transactivates CXCR3 in Th1 cells and CTL cells to mediate migration into sites of inflammation [95]. Although the expression of CXCR3 is highly expressed by CD4⁺ T helper type 1 cells and CD8⁺ T cells, it is also detected on B cells, NK and NKT cells and results in migration towards three chemokines namely, CXCL9/MIG, CXCL10/IP-10, and CXCL11/ITAC [95]. Furthermore, given that known role of Th1 cells in *Mtb* infection, CXCR3 would therefore be a likely candidate involved in cell trafficking. Indeed, CXCL 9-11 expression is induced during *Mtb* infection (Table 1) and expression is localized within TB granulomas [49, 96]. Given that IFN γ is known to be a potent inducer of CXCR3 ligands [95], it is not surprising that the timing of induction of CXCL9-11 mRNA in vivo in the murine lung during *Mtb* infection correlates well with IFN γ mRNA induction in the lung [14]. In addition, other proinflammatory cytokines such as TNF- α can synergize with IFN γ to induce expression of these chemokines [95], while IL-17 made by memory Th17 cells can independently drive expression of CXCR3 ligands in *Mtb* recall responses [97]. Accordingly, CXCR3 expressing T cells are found in *Mtb*-infected NHP lungs and BAL [49] as well as *Mtb*-infected mouse lungs [97]. Further CXCR3+ cells were found in solid or caseous granulomas localized to regions expressing CXCL9, CXCL10, and CXCL11 chemokines within *Mtb*-infected NHP lung [96].

To investigate the mechanistic role of CXCR3 in response to *Mtb* infection, CXCR3KO mice on the C57BL/6J background were infected with low doses of aerosolized *Mtb* infection and did not show any defects in bacterial control; however, tubercle granuloma formation was impaired with associated decrease in the number, size, and density of granulomas in the lung [98]. Similarly, CXCR3 deficient mice on the BALB/c genetic background also did not exhibit higher bacterial burdens in the lung or spleen [99]. Despite these results, CXCR3 is still a dominant chemokine receptor expressed by Th1 cells during *Mtb* infection in mouse [97] and NHPs [49] and CXCL10 protein was significantly increased in patients with active TB [100]. Therefore, it is likely that CXCR3 expression on T cells mediates T cell migration to the lung in response to the inflammatory chemokines, and that in its absence, other chemokine receptor-ligands mediate this function. Furthermore, higher expression of CXCL9 was found to differentiate disease severity in human TB patients [101-102] and a new potential protective SNP in the promoter of CXCL10 has been reported [103], suggesting that despite the redundancy seen in the absence of CXCR3 in vivo in mice models, CXCR3 receptor and its ligands likely play important roles in human TB.

2.2.3 CXCR5—CXCL13, the only ligand to CXCR5, is a homeostatic chemokine expressed constitutively in secondary lymphoid organs [104] and produced by follicular dendritic cells and stromal cells for the specific localization of B cells and Tfh cells within the lymphoid follicle [105-109]. Recently, CXCL13 expression has been detected in peripheral non lymphoid tissues and associated with inflammation seen in conditions such as chronic obstructive pulmonary disease, rheumatoid arthritis and TB [110-112]. CXCL13 is upregulated during an *Mtb* infection (Table 2) [14] and expression is localized specifically within lymphoid follicles containing B cells adjacent to the granuloma [74, 76, 113]. In the absence of CXCL13, integrity of the B cell follicle and formation of the granuloma is severely compromised [76]. Furthermore, B cells isolated from *Mtb* infected murine lungs expressed CXCR5 and migrated to CXCL13 in vitro [114], suggesting that both T cells and B cells expressing CXCR5 may be important during TB. Accordingly, mice deficient in CXCL13 are more susceptible to *Mtb* low dose aerosol infection, due to defects in correct T cell localization within the TB granulomas leading to decreased macrophage activation and mycobacterial control [76]. No doubt future research will be focused on delineating the specific role of CXCR5 on T cells and B cells in formation of lymphoid structure formation within TB granulomas and their specific role in mycobacterial control.

3. Conclusions

As discussed in this review, studies in the past decade have described chemokines that are induced in the lung following *Mtb* infection, identified immune cell types that express the relevant receptors and described redundant and non-redundant functions for these molecules in *Mtb* infection. However, much still remains to be learned about how these chemokines function together to recruit and orchestrate the temporal and spatial localization of immune cells within the lung to form the tubercle granuloma and mediate bacterial control. Current data suggests a model where inflammatory chemokines are induced early in the lung following *Mtb* infection and recruit innate immune cells such as neutrophils and monocytes in an early wave, likely through receptors CCR2/5 and CXCR1/CXCR2 (Figure 2). In the meanwhile, lung DCs carrying *Mtb* upregulate expression of CCR7 and migrate in response to homeostatic chemokines expressed within lymphoid organs to polarize T cells into cytokine producing cells. Subsequent to activation in lymphoid organs, T and B cells upregulate chemokine receptors such as CXCR3, CCR5 and CCR6, respond to inflammation induced chemokines in the lung and accumulate in the *Mtb*-infected lung (Figure 2). There is emerging evidence that upon accumulation of immune cells in the lung, chemokines such as CXCL13 and CCL19 may then mediate correct spatial localization of immune cells to

form granulomas and mediate mycobacterial control. A decade of research using chemokine deficient mice models has shown that expression of multiple chemokine receptors on immune cells confers an advantage to the host, since lack of one or more chemokine-chemokine receptor pathway is mostly compensated for by other chemokine pathways. Despite this knowledge, it is not known whether other factors such as age, immune-suppression or co-infections affects the expression of chemokines and chemokine receptors and if it impacts redundancy associated with the chemokine pathways. Furthermore, the recent association of several chemokine receptors and ligands with disease severity and TB risk suggests that chemokine expression may likely be used as biomarkers in TB. In addition, the knowledge gained about chemokines from these studies will no doubt be harnessed to improve recruitment of protective T cells to the lung in future vaccine design against TB. These future studies will be critical in designing improved diagnostics, therapy and vaccines against TB.

Biography

Shabaana Khader received her PhD in Biotechnology from Madurai Kamaraj University, India where she studied host-pathogen interactions during the mycobacterial disease, leprosy. Dr. Khader then carried out her Post-doctoral training at the Trudeau Institute, NY, where she continued studying host immune responses to another mycobacterial disease, tuberculosis. During her stay at the Trudeau Institute, Dr. Khader demonstrated a critical role for the cytokine Interleukin-17 in vaccine-induced immunity to tuberculosis as well as described seminal roles for IL-12 cytokines in tuberculosis. Dr. Khader then joined the University of Pittsburgh in 2007 as Assistant Professor in the Department of Pediatrics where her lab continues to study the role of cytokines in immunity to intracellular pathogens such as *Mycobacterium tuberculosis* and *Francisella tularensis*. For her accomplishments, Dr. Khader received a Pathway to Independence Award from NIH, Young Investigator Award from the International Cytokine Society and the Pfizer-Showell award from the American Association of Immunologists. Dr. Khader is also an Associate Editor of the Journal of Immunology, Academic Editor of Plos One, member of the American Association of Immunologists Committee on Women in Science and on the editorial Board of the Journal of Infectious Diseases and Immunity and Infection.

Samantha Slight received her BS in Microbiology from Brigham Young University, UT in 2008 and is currently a Ph.D student with Shabaana Khader at the University of Pittsburgh studying the role of chemokine receptors in immunity to tuberculosis.

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Figure 1. Timing of accumulation of immune cells in the murine lung following *Mtb* infection

Innate cells such as neutrophils, $\gamma\delta$ T cells, DCs and macrophages likely respond to early induction of chemokines and accumulate in the *Mtb*-infected lung (a). Following initiation of adaptive immune responses, cytokine-producing CD4⁺, CD8⁺ T cells as well as B cells then respond to appropriate chemokines induced in the lung and accumulate to mount protective immune responses against *Mtb* infection (b). * indicates the timing of arrival of different immune cells to the lung following *Mtb* infection.

Figure 2. Proposed model describing the role of chemokines in cellular recruitment, formation of TB granulomas and host defense during *Mtb* infection

Following *Mtb* infection, alveolar macrophages are likely the first cells to uptake *Mtb* and produce chemokines, likely followed by other chemokine production by other non immune cells such epithelial cells and fibroblasts. This chemokine cascade likely results in early recruitment of innate immune cells such as neutrophils and monocytes, likely through receptors CCR2/5 and CXCR1/CXCR2. In the meanwhile, lung DCs carrying *Mtb* upregulate expression of CCR7 and migrate in response to homeostatic chemokines expressed within lymphoid organs to polarize T cells into cytokine-producing cells. Subsequent to activation in lymphoid organs, activated T and B cells upregulate chemokine receptors such as CXCR3, CCR5 and CCR6, respond to inflammation induced chemokines in the lung to accumulate in the *Mtb*-infected lung. There is emerging evidence that upon accumulation of immune cells in the lung, chemokines such as CXCL13 and CCL19 may then mediate correct spatial localization of immune cells to form granulomas and mediate mycobacterial control.

Table 1

Chemokines and their corresponding chemokine receptors.

CC-Receptors	CC-Ligands
CCR1	CCL3,5,7,8,13,14,15,16,23
CCR2	CCL2,7,8,12,13,16
CCR3	CCL5,7,8,1,13,15,24,26,28
CCR4	CCL17,22
CCR5	CCL3,4,5,8
CCR6	CCL20
CCR7	CCL19, 21
CCR8	CCL1
CCR9	CCL25
CCR10	CCL27, 28

CXC-Receptors	CXC-Ligands
CXCR1	CXCL6,8
CXCR2	CXCL1,2,3,5,6,7,8
CXCR3	CXCL9,10,11
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL11, 12

Table 2

Fold induction of chemokine and chemokine receptor genes in the murine lung at day 12 (D12), day 15 (D15) and day 21 (D21) following *M.tuberculosis* infection [14].

Symbol	Gene Name	D12		D15		D21	
		Fc	FDR	Fc	FDR	Fc	FDR
CXCL1	chemokine (C-X-C motif) ligand 1	1.70	0.04	4.58	0.00	118.27	0.00
CXCL2	chemokine (C-X-C motif) ligand 2	1.10	0.70	2.06	0.02	29.73	0.00
CXCL3	chemokine (C-X-C motif) ligand 3	2.73	0.00	3.86	0.00	36.53	0.00
CXCL5	chemokine (C-X-C motif) ligand 5	19.07	0.00	9.47	0.00	192.71	0.00
CXCL9	chemokine (C-X-C motif) ligand 9	2.78	0.00	93.97	0.00	2134.40	0.00
CXCL10	chemokine (C-X-C motif) ligand 10	1.39	0.13	20.54	0.00	357.38	0.00
CXCL11	chemokine (C-X-C motif) ligand 11	1.00	1.38	1.99	0.02	86.67	0.00
CXCL12	chemokine (C-X-C motif) ligand 12	1.05	1.11	1.16	0.64	2.09	0.05
CXCL13	chemokine (C-X-C motif) ligand 13	1.40	0.16	1.43	0.19	42.89	0.00
CXCL14	chemokine (C-X-C motif) ligand 14	-1.04	1.17	1.24	0.41	1.11	0.91
CXCL15	chemokine (C-X-C motif) ligand 15	-1.16	0.93	-1.12	0.94	-1.12	1.25
CXCL16	chemokine (C-X-C motif) ligand 16	-1.25	0.90	-1.07	1.15	3.82	0.01
CXCL17	chemokine (C-X-C motif) ligand 17	-1.02	1.34	-1.01	1.17	1.99	0.05
CCL1	chemokine (C-C motif) ligand 1	1.00	1.09	1.05	1.33	4.21	0.02
CCL2	chemokine (C-C motif) ligand 2	1.51	0.07	4.45	0.00	232.36	0.00
CCL3	chemokine (C-C motif) ligand 3	-1.28	0.74	1.33	0.29	61.40	0.00
CCL4	chemokine (C-C motif) ligand 4	1.29	0.21	2.04	0.01	147.32	0.00
CCL5	chemokine (C-C motif) ligand 5	-1.30	0.63	1.24	0.45	8.95	0.00
CCL6	chemokine (C-C motif) ligand 6	1.10	0.69	1.23	0.50	1.25	0.62
CCL7	chemokine (C-C motif) ligand 7	1.12	0.59	2.58	0.01	108.37	0.00
CCL8	chemokine (C-C motif) ligand 8	1.82	0.02	3.97	0.00	61.61	0.00
CCL9	chemokine (C-C motif) ligand 9	1.20	0.51	1.41	0.14	3.76	0.01
CCL11	chemokine (C-C motif) ligand 11	-1.24	0.68	1.60	0.09	2.06	0.07
CCL12	chemokine (C-C motif) ligand 12	2.81	0.00	3.13	0.00	69.50	0.00
CCL17	chemokine (C-C motif) ligand 17	1.72	0.04	1.52	0.12	1.77	0.11

Symbol	Gene Name	D12		D15		D21	
		Fe	FDR	FC	FDR	FC	FDR
CCL19	chemokine (C-C motif) ligand 19	-1.44	0.45	1.41	0.25	11.09	0.00
CCL20	chemokine (C-C motif) ligand 20	1.00	1.09	1.00	1.09	75.02	0.00
CCL21A	chemokine (C-C motif) ligand 21a	-1.07	1.15	1.04	1.03	-1.11	1.20
CCL22	chemokine (C-C motif) ligand 22	1.44	0.10	1.31	0.35	3.48	0.01
CCL24	chemokine (C-C motif) ligand 24	1.00	1.09	1.00	1.09	1.00	1.21
CCL25	chemokine (C-C motif) ligand 25	1.16	0.55	1.18	0.64	-1.09	1.29
CCL27A	chemokine (C-C motif) ligand 27A	-1.03	1.26	-1.19	0.80	-2.58	0.09
CCL28	chemokine (C-C motif) ligand 28	1.07	0.84	1.01	1.08	1.08	0.78
XCL1	chemokine (C motif) ligand 1	1.16	0.51	1.76	0.02	3.62	0.01
CX3CR1	chemokine (C-X3-C) receptor 1	-1.37	0.56	-1.18	0.74	2.34	0.03
CXCR2	chemokine (C-X-C motif) receptor 2	1.35	0.26	1.00	1.09	1.63	0.29
CXCR3	chemokine (C-X-C motif) receptor 3	-1.09	1.08	1.30	0.41	29.63	0.00
CXCR4	chemokine (C-X-C motif) receptor 4	-1.23	0.82	1.06	1.00	-1.10	1.22
CXCR5	chemokine (C-X-C motif) receptor 5	-1.44	0.48	-1.07	1.21	1.18	0.92
CXCR6	chemokine (C-X-C motif) receptor 6	1.19	0.46	1.17	0.54	24.26	0.00
CXCR7	chemokine (C-X-C motif) receptor 7	1.21	0.32	1.20	0.35	-1.07	1.41
CCR1	chemokine (C-C motif) receptor 1	2.87	0.00	2.03	0.03	9.53	0.00
CCR2	chemokine (C-C motif) receptor 2	-1.28	0.72	1.02	1.20	3.34	0.01
CCR3	chemokine (C-C motif) receptor 3	1.00	1.09	1.00	1.09	1.00	1.21
CCR4	chemokine (C-C motif) receptor 4	1.00	1.09	1.00	1.09	1.00	1.21
CCR5	chemokine (C-C motif) receptor 5	1.10	0.47	1.22	0.43	16.65	0.00
CCR6	chemokine (C-C motif) receptor 6	-1.22	0.83	-1.40	0.43	-1.09	1.44
CCR7	chemokine (C-C motif) receptor 7	-1.15	0.94	1.41	0.12	2.46	0.03
CCR8	chemokine (C-C motif) receptor 8	1.00	1.09	1.00	1.09	1.11	1.18
CCR9	chemokine (C-C motif) receptor 9	1.09	0.44	2.36	0.00	5.52	0.00
CCR10	chemokine (C-C motif) receptor 10	1.00	1.09	1.00	1.09	1.00	1.21
XCRI	chemokine (C motif) receptor 1	-1.09	1.22	-1.02	1.39	3.13	0.01
CX3CR1	chemokine (C-X3-C) receptor 1	-1.37	0.56	-1.18	0.74	2.34	0.03