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Review

Chemokines in tuberculosis: The good, the bad and the ugly

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ABSTRACT

Mycobacterium tuberculosis (*Mtb*) infects about one-third of the world's population, with a majority of infected individuals exhibiting latent asymptomatic infection, while 5–10% of infected individuals progress to active pulmonary disease. Research in the past two decades has elucidated critical host immune mechanisms that mediate *Mtb* control. Among these, chemokines have been associated with numerous key processes that lead to *Mtb* containment, from recruitment of myeloid cells into the lung to activation of adaptive immunity, formation of protective granulomas and vaccine recall responses. However, imbalances in several key chemokine mediators can alter the delicate balance of cytokines and cellular responses that promote mycobacterial containment, instead precipitating terminal tissue destruction and spread of *Mtb* infection. In this review, we will describe recent insights in the involvement of chemokines in host responses to *Mtb* infection and *Mtb* containment (the good), chemokines contributing to inflammation during TB (the bad), and the role of chemokines in driving cavitation and lung pathology (the ugly).

1. Introduction

Tuberculosis (TB), caused by infection with *Mycobacterium tuberculosis* (*Mtb*), is estimated to affect one-third of the world's population. The majority of infected individuals develop asymptomatic latent TB, while ~5–10% of latently infected individuals will progress to active pulmonary TB (ATB), resulting in about 9 million new cases of TB and 1.4 million deaths per year [1]. The long drug treatment regimes, the relative inefficacy of the current TB vaccine, in addition to the increase in drug-resistant TB cases [1], stresses the importance of understanding host immune responses that mediate *Mtb* control. The past two decades have broadened our understanding of the immune mechanisms required for *Mtb* containment and delineated that the key processes regulating TB control or disease exacerbation involve the recruitment of host immune cell populations into the lung. This process is governed by adhesion molecules and by chemoattractant cytokines or "chemokines", a family of small proteins, which, upon binding to membrane G protein-coupled receptors, guide the gradient-driven migration of leukocytes [2]. Chemokines are classified into the CXC-, CC-, C- and CX3C- subfamilies according to the arrangement of four conserved cysteine residues, which are important for maintenance of their tridimensional structure [2]. A recent review has described the general structure of chemokines and their overall functions in TB [3]. In this review, we have specifically focused on chemokines and their effector mechanisms that contribute to pulmonary control of *Mtb* infection. In addition, we will discuss the importance of chemokines in the establishment of a balance between proinflammatory and anti-inflammatory mediators during TB that may result in improved *Mtb* control or exacerbated disease outcomes.

2. Role of chemokines in mediating *Mtb* control (the good)

Over the past two decades, the availability of animal models of TB, in addition to human studies, has shed light on several key chemokine-driven immune mechanisms mediating *Mtb* control [4]. *Mtb* reaches the lower airways of the lung via inhalation of 3–5 μm droplet nuclei, generated during coughing or sneezing. Upon entry into the lung, mycobacteria are taken up by alveolar macrophages, where *Mtb* replicates while inhibiting macrophage killing mechanisms [5]. Despite this, infected macrophages actively secrete chemokines and cytokines, resulting in the recruitment

Abbreviations: APC, antigen presenting cell; ATB, active tuberculosis; CD, cluster of differentiation; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C chemokine receptor; DC, dendritic cell; ESAT-6, early secretory antigenic target 6 kDa; iNKT, invariant natural killer T cell; IL, interleukin; LN, lymph node; miR, micro RNA; MMP, matrix metalloproteinase; Mtb, *Mycobacterium tuberculosis*; NHP, non-human primate; NK cells, natural killer cells; SLO, secondary lymphoid organ; TB, tuberculosis.

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and activation of several immune cell populations to the lung [5]. Indeed, in the mouse model of low dose aerosol infection, around day 12 post-infection there is an early influx of innate cells into the lungs, including γδ T cells, NK cells, monocyte-derived macrophages, dendritic cells and neutrophils [6]. It is possible that distinct chemokines govern the specific recruitment of these diverse immune cells to the lung. In particular, increased expression of the chemokines CXCL-3 and CXCL-5 is observed as early as day 12 after infection [6], and this correlates with the early influx of neutrophils and NK cells, which likely express the receptor CXCR2. In addition, lung epithelial cells can directly sense *Mtb* and produce chemokines, resulting in a potentiation of immune cell recruitment. In response to *Mtb* stimulation, CCL-2 and CXCL-8 are produced by alveolar epithelial cells and by human bronchial epithelial cells [7,8]. In addition, in the mouse model of *Mtb* infection, following TLR-2 ligation, the lung epithelium has been described to secrete CXCL-5, which, signaling through CXCR2, can increase neutrophil recruitment [9]. Despite the accumulation of these innate immune cells, *Mtb* continues to grow exponentially over the first 2–3 weeks following infection [6]. Thus, activation of adaptive immunity and recruitment of effector T cells into the lung is required for bacterial burden control [10]. The priming of T cells is initiated by dendritic cells (DCs), primary antigen presenting cells (APCs) that serve as a direct link between the innate branch of the immune response and the adaptive response [11].

Lung resident DCs can take up live *Mtb* within the lungs and transport them to the lung-draining mediastinal lymph nodes, where they were thought to serve as APCs [12]. Migration of DCs from the lungs to the mediastinal lymph nodes is governed by chemokine-receptor interactions, and occurs around day 14 post-infection in the mouse model of TB [12]. Uptake of *Mtb* by DCs leads to the upregulation of CCR7 expression [13], which guides the cells to the mediastinal lymph node following a gradient of the homeostatic chemokines CCL-19 and CCL-21 [12]. CCL-21 is expressed by the lymphatic endothelium, directing the initial migration of DCs, while CCL-19 and CCL-21 are expressed by lymph node resident cells. Importantly, mice lacking CCR7 have an impaired ability to migrate to the draining lymph nodes, resulting in delayed priming of *Mtb*-specific T cells [14]. Recently, it has come to light that the cell populations that become infected and carry antigen to the lymph node, and those that directly prime the T cells, are distinct. Indeed, infected $CCR2^+$ inflammatory monocytes are important for antigen delivery from the lung to the draining lymph nodes, where they release soluble antigen that can be taken up and presented by resident lymph node DCs [15,16]. Subsequent recognition of *Mtb* antigens by naïve T cells bearing specific T cell receptors, in the presence of costimulatory signals and adequate cytokines in the microenvironment leads to the activation, proliferation and differentiation of naïve T cells into effector cells [17].

While *Mtb* actively replicates in the lung, induction of inflammatory chemokines ultimately results in the recruitment of newly activated effector T cells from the periphery. T cells that exit the lymph node are able to enter the lung via the circulation through ligation of surface endothelial receptors that are upregulated in response to inflammation. Several chemokines and their cognate receptors have been associated with T cell migration into the lung during TB. $CD4^+$ and $CD8^+$ T cell activation and differentiation in the lymph node is accompanied by changes in surface chemokine receptor expression and the corresponding alteration of their migratory capacity. Upon commitment to the Th1 subset, the main $CD4^+$ T cell subset implicated in *Mtb* control, effector T cells upregulate the chemokine receptors CXCR3 and CCR5 [18,19]. It is thought that this is directly related to their recruitment into the infected lung, as the ligands for these receptors, CXCL-9, CXCL-10 and CXCL-11 for CXCR3 and CCL-3, CCL-4, CCL-5 and CCL-8 for CCR5, are upregulated in *Mtb*-infected mouse [6] and NHP lungs [20]. Several mechanistic studies have addressed the requirement for CXCR3 and CCR5 expression on T cells [21,22], providing evidence that there is significant redundancy in the expression of these inflammatory chemokines and their receptors on the recruitment of *Mtb*-specific T cells to the lung. Human studies have shown associations between mutations in CCL-2 and CCL-5 and pulmonary TB [23–25], suggesting that despite the redundancy observed in animal models, these chemokines may have defined roles to play in human TB.

Upon entry into the lung parenchyma, however, proper *Mtb* containment is dependent on the correct localization of effector T cells in apposition to *Mtb*-infected macrophages. In recent years, several reports have demonstrated the expression of homeostatic chemokines, which are commonly expressed in secondary lymphoid organs (SLOs), in *Mtb*-infected lungs [6,26]. Such chemokines, including CCL-19, CCL-21, CXCL-12 and CXCL-13, drive the organization of lymphoid follicles in SLOs and in the periphery [26]. These organized lymphoid and stromal aggregates, known as ectopic lymphoid follicles, have been reported in conditions of chronic infection and inflammation [27]. Interestingly, during *Mtb* infection in mice, non-human primates and humans, CXCR5-expressing $CD4^+$ T cells also accumulate in the lungs, within ectopic lymphoid follicles [28]. Importantly, these $CXCR5^+$ $CD4^+$ T cells produce high levels of proinflammatory cytokines and upon accumulation in the lung, respond to CXCL-13 likely produced by stromal cells early during infection, and localize near *Mtb*-infected macrophages to mediate *Mtb* control [28]. Accordingly, both CXCR5- and CXCL13-deficient mice lacked the formation of ectopic lymphoid follicles and exhibited decreased control of *Mtb*, thus projecting the non-redundant role for CXCR5–CXCL-13 axis in TB. CXCR5 deficiency resulted in localization of $CD4^+$ T cells around blood vessels in the *Mtb*-infected lungs, forming perivascular cuffs indicative of their inability to localize in apposition to infected macrophages [28]. Therefore, not only is the timely induction of chemokine-mediated recruitment of T cells to the lung critical for *Mtb* control, but emerging evidence suggests that chemokines also play a critical role in the precise positioning of *Mtb*-specific T cells within the lung parenchyma for maximal *Mtb* control. Indeed, early vaccine-induced production of CXCL-9, CXCL-10 and associated recruitment of CXCR3-expressing T cells is beneficial in vaccine-induced protection against *Mtb* challenge [29]. In addition, vaccine strategies that induce early CXCL-13 production to enhance and improve early T cell localization near *Mtb*-infected macrophages can be harnessed for vaccine design against TB [30].

Together, there is accumulating evidence that chemokines induced in response to *Mtb* infection effectively mediate DC trafficking to the LNs, recruitment of activated T cells to the lung and correct localization of T cells within the lung parenchyma to mediate optimal *Mtb* control. However, although these chemokine-dependent processes mediate control of *Mtb* growth, they often do not completely eliminate the bacteria (Fig. 1). Further understanding of the mechanisms that lead to *Mtb* containment will not only allow the better development of novel therapies against TB, but will be of particular relevance for vaccine and adjuvant design (Table 1).

3. Chemokines mediate inflammation during TB (the bad)

The aforementioned mechanisms of TB containment rely on a precise site and time-specific upregulation of chemokines and their receptors. However, numerous factors can shift the balance to limited containment or pathology. Indeed, the nonresolving immune activation that occurs in chronic diseases such as TB can lead to tissue damage and pathology. Given that maintenance of lung architecture is essential for adequate organ function, unrestricted inflammation at this site is associated with respiratory

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