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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).

Keywords

Chemokines; Mycobacterial infections; lung

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

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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, have 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 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 a line of 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 into the lung, 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 (Figure 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.

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 failure and increased mortality in TB patients [31]. Identification of the factors leading to exacerbated inflammation within TB lungs will enable the development of new therapies for TB.

A neutrophil-associated human blood transcriptional signature was seen in patients with ATB [32], and neutrophils were identified as the predominant cell infected with replicating *Mtb* in ATB patients [33]. In mice, different inbred strains vary in their susceptibility to *Mtb*. In particular, resistant strains such as C57BL/6 form smaller lung lesions mostly comprised of lymphocytes and macrophages [34]. Susceptible strains, including C3H and I/St mice, however, form more diffuse and less organized lesions, with increased neutrophil infiltration [35–37]. Emerging evidence suggests that the accumulation of neutrophils during TB is

mediated by interaction between the chemokine CXCL-5 and its receptor CXCR2, specifically expressed on neutrophils. Dorhoi et al [38] recently reported that the increased susceptibility to *Mtb* challenge observed in miR-223-deficient mice, could be reversed by CXCL-2 blockade, CCL-3 and IL-6 neutralization, or by neutrophil depletion [38], underscoring the importance of tightly regulated inflammation for host survival. Importantly, in a high dose infection model, mice deficient in CXCR2 or CXCL-5, had a prolonged survival and decreased lung pathology in comparison to their wild type counterparts [9]. That the CXCL-5 upregulation was mediated by TLR-2 signaling [9], suggests that *Mtb* may have evolved ways to harness neutrophil recruitment to enhance inflammation and disease severity in the host. In unraveling the mechanisms by which neutrophils mediate lung pathology in TB, we recently showed that in NHPs and patients with ATB, increased pulmonary pathology correlated with increased neutrophil accumulation and expression of neutrophil-associated products such as S100A8/A9 proteins [39]. The effect of S100A8/A9 proteins in promoting neutrophil and monocyte migration occurs through the induction of proinflammatory chemokines such as CXCL-1, and the upregulation of integrins such as CD11b on neutrophils. Thus, in ATB patients and diversity outbred (DO) mice, which constitute a new model of the genetic variability, lung damage score correlated well with levels of S100A8/A9 and CXCL-1 protein levels [39].

Consistent with these findings, several recent papers have linked increased levels of specific neutrophil chemokines with ATB in patients, suggesting their potential as immunological markers for TB and for treatment responsiveness monitoring [40, 41]. Indeed, CCL-3, CXCL-8 and CCL-2 expression was upregulated in neutrophils isolated from patients with pulmonary TB in comparison to healthy controls, and following in vitro *Mtb* infection [42]. In addition, the CCL-2 2518G allele, which results in exacerbated CCL-2 secretion [43], and combinations of single-nucleotide polymorphisms in the CCL-5 promoter [24] have been associated with TB in human populations. Other chemokines and cytokines have also been shown to be elevated during pulmonary ATB. In particular, Yu et al demonstrated elevated IL-2, CXCL-10, CXCL-11 and CXCL-12 in patients with ATB and increased levels of CCL-1, CCL-21 and IL-6 in patients with tuberculous pleuritis [44]. Further, chemokine determination in the sera of ATB patients before and after treatment could serve as a correlate of treatment efficacy, as a decrease in the serum levels of CXCL-8, CXCL-9 and CXCL-10 was observed in patients with ATB following antibiotic treatment completion [45]. In addition, genetic studies have revealed an association of the 135G/A polymorphism at the level of the CXCL-10 allele and TB in a Chinese population [46]. Taken together, these findings demonstrate an emerging role for chemokines in mediating neutrophil accumulation and perpetuating inflammation during TB (Figure 2), which could be harnessed as novel therapeutic regimes and utilized as novel biomarkers.

4. Role of chemokines in cavitary TB (The ugly)

A hallmark of active TB is the development of pulmonary cavities, which are thought to harbor high levels of replicating bacteria and constitute a contributing source of disease transmission [47]. In humans, cavitation usually occurs in the lung apices and requires dissemination of bacteria from the lung bases, where the infection typically originates [48]. The current paradigm is that cavitary TB is a product of ineffective granuloma formation,

with necrosis development, liquefaction of the necrotic areas and subsequent connection of the mycobacteria-rich granulomatous content and the airways [47]. Caseation is an active process led forward by host and bacterial factors, and potentiated by unrestricted inflammation. Indeed, immune mediators such as reactive oxygen species and reactive nitrogen intermediates [49, 50], cytokines and pro-apoptotic receptors [51, 52] can induce macrophage necrosis and/or apoptosis, releasing the cell contents that form the center of the caseous granuloma. Given that lung structure is maintained by tightly bound collagen fibrils, active processes involving hydrolase activation are required for destruction of the lung parenchyma and subsequent cavitation. Amongst the hydrolases that participate in tissue destruction, matrix metalloproteinases (MMPs) play a central role in cavitation [48].

MMPs are a family of zinc-containing proteases with a range of substrate specificities and sources that play a key role in extracellular matrix degradation. In addition, MMPs can modulate cytokine and chemokine activity by cleavage, either inactivating them or potentiating their biological activity. Importantly, MMP activity is implicated in TB progression both in animal models and in humans with ATB. Indeed, in a zebrafish model of *M. marinum* infection, epithelial cells upregulated MMP-9 expression in response to *Mtb*-derived ESAT-6, and enhanced macrophage recruitment, increasing granuloma growth and mycobacterial proliferation [53]. In addition, mice overexpressing the human MMP-1 gene under the control of the macrophage-specific promoter for scavenger receptor A had increased lung matrix destruction during *Mtb* infection [54]. Further, use of the broad spectrum MMP inhibitor BB-94 decreased *Mtb* burden, reduced granuloma size and leukocyte recruitment [55, 56]. MMP-9-deficient mice had lower lung macrophage recruitment with less well-formed granulomas and reduced bacteria [57]. In animal models where cavitation occurs in response to TB, such as the guinea pig [58], and rabbit [59], activation of proteolytic enzymes is associated with lung damage and cavitation [60, 61]. Recently, elevated sputum levels of several MMPs were reported in patients with TB, and MMP-1 and -3 concentrations positively correlated with TB severity [62]. Interestingly, MMP levels at the time of diagnosis negatively correlated with responsiveness to treatment [62].

In addition to driving direct tissue destruction, MMPs can exert their functions on chemokines, thereby altering leukocyte recruitment. Chemokine digestion by MMPs can have several outcomes, ranging from functional inactivation, to the generation of antagonistic variants and to potentiation of chemotactic activity [63]. Inactivation of several chemokines has been shown to occur through MMP-mediated cleavage. In fact, MMP-1, -2, -3, -9, -13 and -14 can cleave CXCL-12, rendering it functionally inactive [64]. Given that CXCL-12 is one of the homeostatic chemokines induced in the mouse lung in response to *Mtb* infection [6], MMP digestion could potentially affect ectopic lymphoid follicle formation, thereby altering *Mtb* control. MMP-9 can also inactivate CXCL-4 and CXCL-1 [65], potentially affecting monocyte and neutrophil recruitment. In addition, MMP-8 and MMP-9 can proteolytically inactivate CXCL-9 and CXCL-10, but not CXCL-11 [66], a process that could affect T cell recruitment into *Mtb*-infected lungs. However, given the redundancy between CXCL9, -10 and -11, whether this mechanism substantially affects T cell migration in TB is not known. Another potential outcome of MMP-driven chemokine

proteolysis is the generation of variants that retain receptor binding ability but are unable to mediate chemotaxis. Because they can compete with active chemokines, these variants could serve as chemokine antagonists. For instance, MMP-1 and MMP-3 can cleave CCL-2, generating an antagonist molecule [67] that may affect T cell, neutrophil and NK cell recruitment via CXCR2 during *Mtb* infection. MMP-3, in addition, can generate a CCL-8 antagonist [67], which could impact $CCR5^+$ cell recruitment. In addition, MMPs can potentiate chemokine activity. For instance, MMP-9 can process CXCL-8 (IL-8), leading to an increase in chemotactic activity [65]. CXCL-5, which signals through CXCR2, can also be processed by MMP-9 into a more active molecule [68]. Given that neutrophils are a main source of MMP-9, this could serve as a positive feedback loop, allowing the perpetuation of an inflammatory response in TB. In vivo, chemokines bind to glycosaminoglycans, which contribute to their stability and to the adequate formation of tridimensional gradients. MMP activity on extracellular matrix proteins can therefore also indirectly affect chemokine activity. For instance, the chemokine CXCL-1 can bind the cell surface-expressed proteoglycan syndecan-1. Interestingly, MMP-7 and MMP-9 can cleave the extracellular domain of syndecan-1, releasing the syndecan-1/CXCL-1 complex [69]. This creates a chemokine gradient that promotes neutrophil infiltration into the alveolar space and can drive further inflammation during acute lung injury [69]. Resonating with these findings, recent reports have shown increased neutrophils in bronchoalveolar lavages from patients with cavitary TB in comparison to non-cavitary TB patients, associated with decreased CXCL-10 and IL-6, which may indicate the failure of adaptive immunity at this stage of disease [70].

The events leading to terminal tissue destruction and cavitation in TB are beginning to be defined, and likely involve a complex interplay between immune activation, protease function and bacterial factors. Given the dual effect of MMPs in chemokine activity, it is likely that an imbalance between chemokine inactivation and potentiation leads to the unrestricted recruitment of immune cells to the site of infection. This, in turn, may promote overwhelming lung inflammation and destruction, contributing to the events ultimately leading to cavity formation and TB spread (Figure 3). Future studies will shed light on the relative contribution of MMP-derived chemokine variants in TB pathology. Of note, mouse models where granuloma necrosis occurs, such as the C3HeB/FeJ strain [71], will be of particular utility for the study of the mechanistic relevance of MMPs and chemokine variants to lung damage.

6. Conclusions

Chemokines play a central role in orchestrating the recruitment of cells into the *Mtb*-infected lung, which contributes to *Mtb* containment. However, under specific conditions, chemokines can also drive the disproportionate inflammation and lung damage that precipitate progression to pulmonary disease as well as cavitation. In humans, the balance between protective and damaging inflammation, as well as the levels of inflammation required for *Mtb* containment may differ. A number of factors, including host genotype, bacterial strain, co-morbidities and nutritional status likely shift the magnitude and nature of mechanisms that permit *Mtb* control. Understanding the nature and the effect of these

interactions between the host, mycobacteria and the environment will be central to the design of effective therapeutic and vaccination strategies.

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Abbreviations

APC	Antigen presenting cell
ATB	Active tuberculosis
CD	Cluster of differentation
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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Highlights

- Chemokines govern cell influx to *Mtb*-infected lungs and are critical for TB control.
- Productive granuloma formation is tightly regulated by lung-secreted chemokines.
- Chemokine dysregulation can shift the balance from protection to inflammation.
- Host, pathogen and environmental factors can impact chemokine secretion in the TB lung.

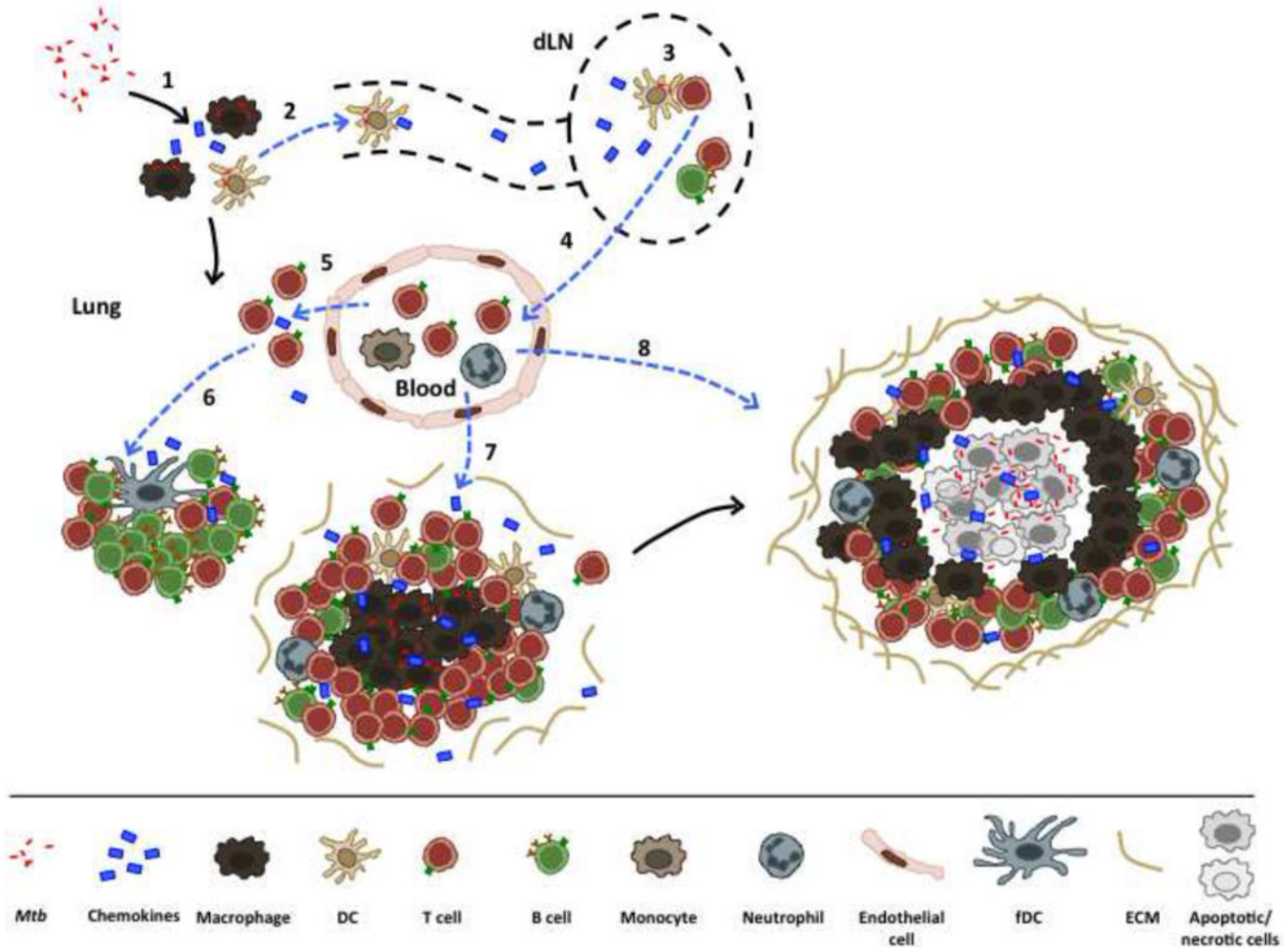

Figure 1. "The good": mechanisms that mediate lung *Mtb* containment

Upon *Mtb* entry into the lungs, alveolar macrophages become infected, leading to secretion of cytokines and chemokines, which drive additional innate cell recruitment (1). Infected DCs migrate into the lung draining lymph nodes (dLN) (2), carrying antigen that can be subsequently taken up by other APCs to activate naïve T cells (3). After activation, T cells (along with B cells) regulate their chemokine receptor expression, which guide their exit from the lymph node (4), homing to the infected lung (5), and subsequent migration. These responses are mediated by differential expression of chemokines, a process that enables ectopic lymphoid follicle formation (6). Additional innate cells, such as monocytes and neutrophils are also recruited into the lung (7). Together, interactions between innate and adaptive cells lead to granuloma formation and *Mtb* containment (8). Dashed blue lines represent chemokine-driven mechanisms.

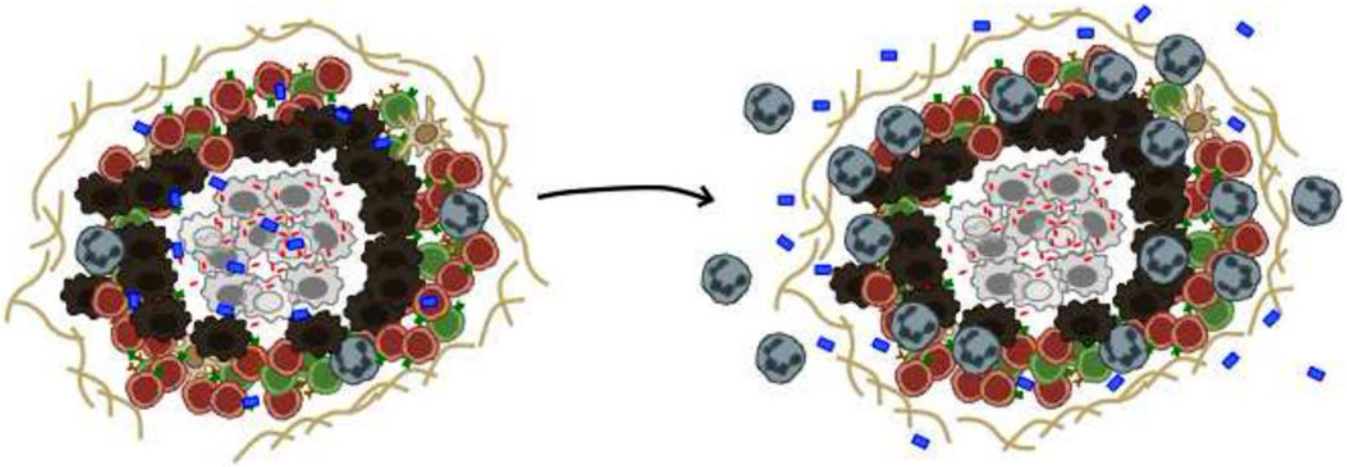

Figure 2. “The bad”: transition to a dysregulated proinflammatory granuloma

An imbalance between anti-inflammatory and pro-inflammatory factors can lead to dysregulated inflammation in TB, a feature of which is accumulation of large numbers of neutrophils in the lungs. Through the secretion of numerous chemokines and molecules, such as S100A8/A9 proteins, neutrophils can perpetuate inflammation during TB.

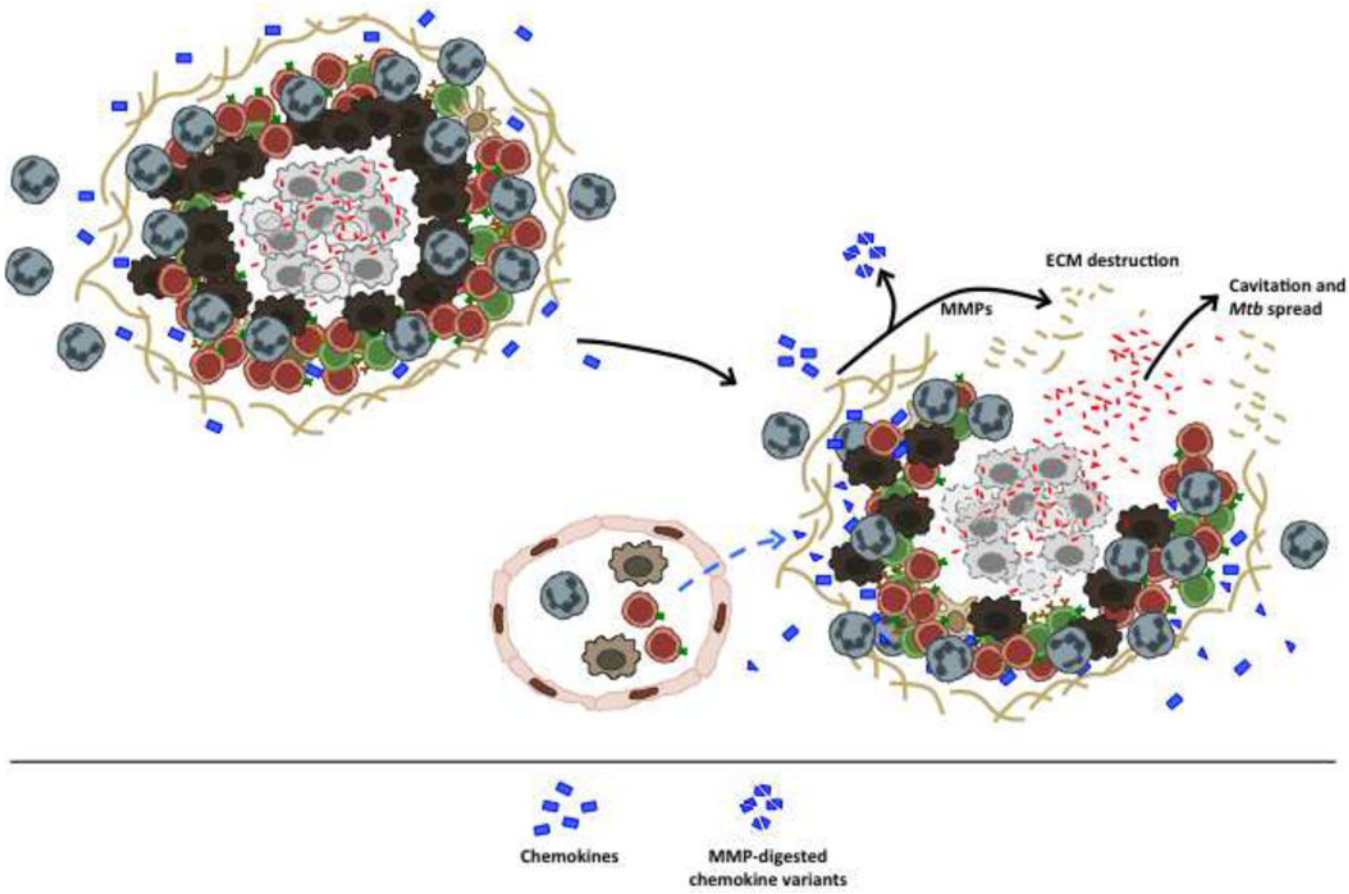

Figure 3. "The ugly": TB necrosis, cavitation and spread

The hallmark of active TB in humans and in several animal models is cavitation, which is a product of persistent inflammation and tissue destruction. Central to this process are matrix metalloproteinases, which can degrade the extracellular matrix that maintains lung structure, and cleave chemokines. These novel chemokine variants can be more or less active than their native counterparts, and their production likely alters the balance of cells that are recruited into the lung. This overwhelming inflammation and matrix destruction communicates *Mtb*-rich granulomas with the airways, facilitating *Mtb* spread.

Table 1

Chemokines and chemokine receptors in TB

Receptor	Ligands	Role in TB	Reference
CCR2	CCL2,7,8,12,13,16	Recruitment of monocytes, DCs, T cells and NK cells.	[72–74]
CCR4	CCL17,22	Recruitment of T cells. May recruit NK cells, macrophages, DCs and basophils.	[75, 76]
CCR5	CCL3,4,5,8	Recruitment of T cells into the lung. May be involved in macrophage, DC and neutrophil migration.	[19, 75, 77]
CCR6	CCL20	iNKT and T cell recruitment.	[78, 79]
CCR7	CCL19,21	DC migration into the draining lymph nodes, recruitment of naïve and central memory cells to lymph nodes, ectopic lymphoid follicle formation.	[12–14, 26, 80]
CXCR1	CXCL6,8	Recruitment of neutrophils.	[81]
CXCR2	CXCL1,2,3,5,6,7,8	Recruitment of neutrophils and NK cells.	[73, 81, 82]
CXCR3	CXCL9,10,11	Recruitment of T cells into the lung.	[6, 18–20]
CXCR5	CXCL13	Ectopic lymphoid follicle formation.	[28]