Adaptive Immune Response in Leprosy

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Introduction

While the innate immune response against leprosy provides the first line of defense against a *Mycobacterium leprae* infection, the subsequent activation of the adaptive immune system is a crucial event in an effective host defense against the intracellular pathogen. Furthermore, the clinical spectrum of leprosy is determined by the type of adaptive immune response that is elicited. Patients mounting strong cell-mediated immune responses develop paucibacillary (PB) disease and those patients with poor cell-mediated responses or predominant humoral responses develop multibacillary (MB) disease.

This chapter will explain more specifically the relevant cell types that are involved in protective and non-protective immune responses. In particular, the chapter discusses cells that are effective in antimicrobial responses—Th1 and Th17 cells, CD1-restricted T cells, and CD8+ cytotoxic cells—and cells that contribute to permissive states of MB disease—Th2 cells, regulatory T cells, suppressor CD8+ T cells, and B cells.

Th1 vs Th2 Responses in Leprosy

T cells expressing CD4 are also known as helper T cells due to their ability to induce other immune mechanisms such as antibody production, macrophage activation, and CD8+ T cell-mediated responses. Decades ago, studies conducted in mice, and later in humans, established that CD4+ T cells populations could be divided into two subsets, designated T-helper 1 (Th1) and T-helper 2 (Th2) cells, based on their cytokine production profile (1). Th1 cells were shown to produce high levels of interleukin-2 (IL-2), interferon-γ (IFN-γ), granulocyte–macrophage colony-stimulating factor (GM-CSF), and IL-3 upon stimulation, while Th2 cells produce IL-3, IL-4, and IL-5.

The relevance of the Th1 vs Th2 paradigm in a spectrum of human disease was first shown by measuring cytokine mRNA expression in leprosy skin lesions. Patients with tuberculoid leprosy (TT), who can restrict the growth of the pathogen, were shown to predominantly express IL-2, lymphotoxin-α, and IFN-γ in their skin lesions (2). In contrast, patients with lepromatous leprosy (LL), who fail to mount an effective cell-mediated immune response against *M. leprae*, exhibited high expression of Th2 cytokines such as IL-4, IL-5, and IL-10 (2), as shown in Figure 1.

The cytokine pattern observed across the leprosy spectrum reflects the distribution of T-cell populations found in leprosy skin lesions. TT skin lesions display a predominance of CD4+ T cells rather than CD8+ T cells, with a CD4:CD8 T-cell ratio >1. In contrast, LL skin lesions exhibit a higher abundance of CD8+ T cells, with a CD4:CD8 T-cell ratio <1 (3, 4, 5, 6). The CD4+ T cells found in TT skin lesions have been shown to secrete high amounts of IFN-γ, while the CD8+ T-cell population found in LL lesions have been characterized as suppressor CD8+ T cells due to their lack of CD28 expression and ability to secrete high levels of IL-4 (7, 8).
TT patients have a robust cell-mediated Th1 immune response via IL-2, IFN-γ, and lymphotoxin that activates macrophages and cytotoxic T lymphocytes (CTL) to kill the intracellular mycobacteria, resulting in a self-limited disease. In contrast, LL patients have a prominent Th2 response via IL-4, IL-5, and IL-10 that facilitates humoral responses and inhibits cell-mediated immune responses through macrophage suppression, resulting in ineffective mycobacterial control and progressive infection.

The Th1 vs Th2 paradigm and its correlation with cell-mediated immunity (CMI) vs humoral immunity can also be observed during reversal reaction (RR) episodes in leprosy patients (see Chapter 2.2). During an episode, an influx of CD4+ T cells in the skin lesion is observed (9), and the analysis of the cytokine profile of MB patients before the onset of RR and at the time of RR diagnosis shows a switch from Th2 to Th1 local cytokine production (Figure 2) (10).

Patients with TT have enhanced immunity against *M. leprae* via the induction of IFN-γ and IL-15, which activate the vitamin D antimicrobial pathway. In contrast, LL patients are unable to control the infection because the humoral response suppresses macrophage activation and T-cell proliferation. RR occurs in MB patients as a result of increased activity of the immune system, which shows a switch from the insufficient Th2 humoral response to the Th1 antimicrobial response.
Cell-Mediated Immunity

TH1 RESPONSES

Th1-type cytokines have been shown to promote the killing of intracellular pathogens and the development of autoimmune responses (11, 12). The cytokines are characterized by the high production of pro-inflammatory cytokines such as IL-2 and IFN-γ. Early studies focused on the role of IL-2 as a major driving factor of Th1 responses in leprosy and attributed the unresponsiveness observed in LL to a deficiency in IL-2 mediated responses (13). It was observed that LL PBMCs failed to produce IL-2 in response to M. leprae (14) and that T cells from LL patients did not express receptors for IL-2 or produce IL-2 in response to M. leprae (15). Even though studies of lepromin injection sites showed an accumulation of cells staining for both IL-2 receptor and IL-2 in both lepromatous and tuberculoid lesions, over time their expression was reduced in the lepromatous late responses while maintained in the tuberculoid lesions (16). However, it was soon observed that even though IL-2 administration could lead to an increase of CMI and the reduction of bacillary load in lesions (17, 18, 19), this response was not specific to M. leprae and could not modify the selective anergy to the bacilli observed in lepromatous patients (19, 20, 21).

The activation of a Th1 CD4⁺ T-cell response with the release of IFN-γ is critical to an efficient immune response against M. leprae infection (2, 22). IFN-γ increases the expression of MHC class I and II, as well as co-stimulatory receptors, increasing M. leprae-antigen presentation by dendritic cells (DCs) (23, 24, 25, 26). The secretion of IFN-γ by M. leprae-specific Th1 CD4⁺ T cells enhances M. leprae antigen presentation and activates the antimicrobial response in leprosy. Subsequently, toll-like receptor (TLR) activation by M. leprae pathogen-associated molecular patterns (PAMPs) activates DCs and increases antigen presentation to M. leprae-specific CD4⁺ and CD8⁺ T cells in the lesion, with cell proliferation and IFN-γ production (2, 7, 27).

IFN-γ is critical for the induction of an antimicrobial activity against mycobacterial infection (28, 29). IFN-γ is crucial for macrophage plasticity, as it induces changes in the behavior of M0 macrophages, which undergo phenotypic modification to become M1 inflammatory macrophages. M1 macrophages produce pro-inflammatory mediators, including cytokines and induced nitric oxide synthase (iNOS). The latter enzyme induces the production of NO, generating free radicals that destroy the bacillus (30, 31, 32). IFN-γ induces the differentiation of monocytes to M1 macrophages, through the induction of jagged 1 (JAG1) expression by endothelial cells. JAG1 expression is restricted to the regions of the granuloma enriched to M1 macrophages in TT lesions. IFN-γ and JAG1 are involved in the endothelial cell instruction of the antimicrobial macrophage response against M. leprae at the site of infection (33). IFN-γ also induces the activation of the vitamin D antimicrobial pathway, which includes the induction of autophagy and antimicrobial peptides (Figure 2) (34, 35). IFN-γ and its downstream vitamin D-dependent antimicrobial genes are preferentially expressed in TT and RR skin lesions, suggesting the role of this cytokine in driving protection in leprosy (34, 36, 37). In addition, IFN-γ can also induce S100A12, an antimicrobial peptide that
can kill *M. leprae in vitro* (Figure 3) (38, 39, 40). Autophagy and antimicrobial peptides are more highly detected in the skin of TT than LL patients.

![Model of innate and acquired antimicrobial activity.](image)

*FIG 3* Model of innate and acquired antimicrobial activity.

*M. leprae* infection in human macrophages triggers the activation of both innate and adaptive immune mechanisms that act together to eliminate the bacteria. Signal-
The innate immune response to mycobacterial infection (see Chapter 6.1) has a role in the induction of IFN-γ production. The TLR activation by \textit{M. leprae}-PAMPs induces both antigen presentation and production of proinflammatory cytokines. The induction of IL-12, IL-15, and IL-18 can enhance both innate and adaptive immunity against \textit{M. leprae} (55, 56, 57). TLR2/1 activation by \textit{M. leprae} lipoproteins induces IL-12 secretion by DCs, leading to the proliferation of \textit{M. leprae}-specific T-cell clones isolated from TT and LL patients (55, 58). The binding of IL-12 by its receptor increases the release of IFN-γ by Th1 CD4\(^+\) T cells (55, 59, 60). The induction of IFN-γ secretion by IL-12 upregulates TLR1/2, CD40L, and CD40 expression, leading to the amplification of the IL-12 production by DC (59, 60, 61). IL-15 is also produced by DCs and macrophages in response to TLR2/1 activation by \textit{M. leprae} and similarly induces IFN-γ production by \textit{M. leprae}-specific CD4\(^+\) T cells (62, 63). IL-12 and IL-15 proteins are upregulated in the granulomas of TT skin lesions as compared to LL skin lesions. As observed for IL-12 and IL-15, IL-18 mRNA expression is higher in lesions of TT patients as compared with LL patients. IL-18 mRNA has been highly detected in the PBMCs of TT vs. LL patients after \textit{M. leprae} stimulation. DCs and monocytes secrete IL-18 in response to \textit{M. leprae} and, subsequently, IL-18 induces IFN-γ production by NK and T cells. The mechanism by which \textit{M. leprae} induces IL-18 secretion is incompletely understood; however, a recent study indicates that \textit{M. leprae}-derived transfer RNA stimulation of TLR8 induces secretion of IL-18 by monocytes/macrophages (64). IL-18 induces early IFN-γ secretion by NK cells and later IFN-γ production by T cells (57). Unlike IL-12, IL-18 is only efficient in inducing IFN-γ secretion in T cells from TT patients, while IL-18 fails to induce IFN-γ secretion by T cells from LL patients (55, 57). This difference may be due to the lower levels of IL-18R expression by T cells of LL patients secondary to the activation of Stat6, which can inhibit IL-18R expression (65, 66). But IL-18 can act in synergy with IL-12 to enhance the ability of T cells from LL patients to produce IFN-γ in response to \textit{M. leprae} (55, 66, 67). IL-12, IL-15, and IL-18 are also able to enhance IFN-γ production from both T cells and NK cells in response to \textit{M. leprae} antigens, with greater IFN-γ secretion from cells of tuberculoid patients as compared to lepromatous patients (57, 62, 68, 69).

The subcutaneous injection of IFN-γ into the lesions of LL patients induces rapid bacillary clearance (70). IFN-γ treatment increases the expression of both class I and II MHC and co-stimulatory receptors in DCs, leading to an increase of \textit{M. leprae} antigen presentation to T cells and the secretion of inflammatory cytokines like IL-12 (59, 60). IFN-γ treatment also upregulates MHC I and MHC II expression in Schwann cells, facilitating the presentation of \textit{M. leprae}-specific antigens to CD4\(^+\) and CD8\(^+\) T cells (71, 72, 73). While the potential of IFN-γ injection as an adjunct therapy for patients with persistent bacillary load despite MDT seems promising, there is evidence that IFN-γ injections can precipitate erythema nodosum leprosum (ENL) (70, 74). In fact, in a small cohort of 10 patients with BL or LL, 60% developed ENL when treated with chronic subcutaneous IFN-γ injections for 6–10 months. The development of IFN-γ induced ENL could be prevented with concomitant therapy with thalidomide, presumably through its effect of reducing TNF-α secretion. Unfortunately, the addition of thalidomide completely negated the enhanced bacillary clearance that the IFN-γ injection provided. Therefore, alternative methods of ENL prevention are necessary before IFN-γ injection can be considered as an effective treatment for patients with persistent \textit{M. leprae} infection.
CD1 PROTEINS AND HUMAN SKIN DENDRITIC CELL SUBSETS

CD1 proteins are evolutionarily conserved MHC class I-like antigen-presenting molecules. The proteins have evolved the ability to present non-peptide lipid and glycolipid antigens to T cells, including headless self-lipids, inflammatory-associated lipid antigens derived from venoms, and those lipids and glycolipids found abundantly in pathogenic mycobacterial membranes and cell walls (75, 76, 77, 78, 79). The human CD1 gene family encodes a type 1 integral transmembrane glycoprotein containing α₁, α₂, and α₃ extracellular domains non-covalently paired with β₂-microglobulin, analogous to MHC class I molecules (80, 81). After biosynthesis in the endoplasmic reticulum, CD1a-d proteins acquire self-lipids and are shuttled to the plasma membrane via the Golgi apparatus route. CD1 molecules are then internalized and sorted into the endocytic pathway where they might capture self- or foreign antigens. Finally, the CD1–ligand complexes cycle back to the cell surface to activate antigen-specific T cells (80, 81, 82, 83).

CD1 proteins are differentially expressed in several immune cells, mainly by professional antigen presenting cells (APCs) such as dendritic cells (DCs) (80, 81). CD1a is most strongly expressed on Langerhans cells (LCs), a resident DC population in the epidermis in skin (84, 85). LCs expressing CD1a have been shown to present the \textit{M. leprae} antigen to T cells (86). In the context of mycobacteria-derived antigens, CD1a presents a didehydroxy form of mycobactin (DDM) to T cells (77). CD1b has the ability to present a diverse set of mycobacterial antigens to T cells, including mycolic acid, lipoarabinomannan (LAM), phosphatidyl-myoinositol mannoside (PIM), and mycobacterial glucose monomycolate (GMM) (75, 76, 87, 88, 89). Of relevance to leprosy, a T-cell clone derived from a TT lesion recognized \textit{M. leprae} LAM in the context of CD1b (76). CD1c recognizes mycobacterial polyketides mannosyl-β-1-phosphomycoketide (MPM) and phosphomycoketide (PM) (90, 91, 92, 93).

Autophagy by LCs bridges an association between antimicrobial activity and CD1a-mediated antigen presentation (94). IFN-γ treatment induces antimicrobial activity in \textit{M. leprae}-infected LCs through autophagy, which facilitates \textit{M. leprae} phagolysosomal degradation and enhances the ability of LCs to present \textit{M. leprae} antigens to CD1a-restricted T cells. The frequency of LCs with LC3⁺ autophagic vacuoles at the site of leprosy infection correlates with the clinical presentation; it is greater in patients with limited, as compared to progressive, disease (94). CD1-restricted T cells can also deviate the humoral immune response to the \textit{M. leprae} LAM antigen by influencing IgG subclass switching and downregulating IgE production (95).

TH17 CELLS IN LEPROSY

While the study of immunology in leprosy has demonstrated the unique role of Th1 cells in CMI in TT and Th2 cells in LL, more recent studies have shown that a variety of adaptive cell types, including Th17 cells, contribute to the host defense against \textit{M. leprae}. Th17 cells are a distinct lineage of helper T cells that have been shown to be important for combating extracellular pathogens.
Human naïve CD4+ T cells differentiate into Th17 cells through exposure to IL-6, IL-1β, TGF-β, and IL-23 (96, 97). Activated Th17 cells characteristically secrete the cytokines IL-17A, IL-17F, and IL-22, which induce epithelial cell production of IL-1β, IL-6, CXCL2, and CXCL8 to attract and activate inflammatory cells at the site of infection (98, 99, 100, 101, 102, 103, 104). IL-17 and IL-22 from Th17 cells are also able to stimulate the secretion of directly antimicrobial peptides, such as defensins and S100A proteins, from epithelial cells (100, 101, 103, 105). Genetic disturbances of Th17 differentiation and function in humans results in susceptibility to chronic infections with a variety of bacterial and fungal pathogens, especially Staphylococcus aureus and Candida albicans (106, 107, 108).

There have been several studies investigating the role of Th17 cells in M. leprae infection, most of which associate Th17 cells with PB forms of the disease. The serum of leprosy patients, regardless of the clinical subtype, have lower concentrations of IL-17A compared to healthy controls, perhaps indicating that the dysfunction of Th17 responses contributes to leprosy pathogenesis (109, 110). Within clinical subtypes of leprosy, serum concentrations of IL-17A are significantly elevated in PB disease vs MB disease. When stimulated with sonicated M. leprae, PBMCs from patients with TT express significantly greater amounts of IL17A, IL17F, IL22, and IL23A mRNA transcripts compared to PBMCs from LL patients (111). This difference is mirrored at the protein level, where PBMCs from TT patients secrete significantly more IL-17A, IL-17F, IL-23, and IL-6 than PBMCs from LL patients in response to M. leprae (111, 112). The increase in Th17 mRNAs and proteins in activated PBMCs from PB patients is due in part to a greater frequency of Th17 cells (113). There are also greater numbers of Th17 cells and Th17 cytokine mRNA and protein secretion in PB lesions as compared to MB lesions (111, 112, 114, 115). This correlation of Th17 responses with the clinical forms of leprosy is similar to that of Th1 cells, indicating an association between Th17 cells and an effective defense against M. leprae.

The reactional states of leprosy (see Chapter 2.2), the hallmarks of which are increased inflammation and cellular infiltration of existing and new lesions, are typically associated with a transient shift in the immune response of the patient towards the Th1 response (10). Interestingly, the characteristic Th17 cytokines IL-17A, IL-17F, IL-22, and IL-26 all act on epithelial cells to produce inflammatory cytokines IL-1β, IL-6, and TNF-α and a variety of chemokines that induce inflammation and promote the recruitment of inflammatory immune cells, suggesting a role for Th17 cells in reactional states of leprosy (100, 101, 102, 116). M. leprae antigen-stimulated PBMCs from patients with RR or ENL have significantly higher levels of IL17A, IL17F, IL23, IL6, and IL21 mRNA transcripts than stimulated PBMCs from patients with TT or LL (112). Furthermore, IL-17A, IL-21, IL-23A, and IL-6 secretion and IL-17A+CD4+ T-cell frequency are significantly greater in M. leprae stimulated PBMCs from patients in reactional leprosy states as compared to non-reactional states. Enhanced secretion of IL-6 and TGF-β by macrophages may account for the increased frequency of circulating Th17 cells during leprosy reactions. Within granulomas, IL-17A and TGF-β are also more abundant in biopsies from RR and ENL patients than from TT and LL patients (112). The association of Th17 cells with PB forms of the disease and the increase in Th17 activity during reactional states of leprosy highlight the fact that patients who are mounting resistance to M. leprae have a greater frequency of Th17 cells both in circulation and resident at the site of infection.
Aside from the ability of Th17 cells to induce inflammation indirectly through bystander epithelial cells, they contribute to host defense against leprosy more directly through the secretion of IL-26, a recently described Th17 cytokine (40, 117) (Figure 4). IL-26 is a cytokine of the IL-10 family that shares structural similarities with pore-forming antimicrobial proteins, including a cluster of cationic residues on one side of the molecule and a cluster of hydrophobic residues on the opposite side (105, 118). Dang et al. found that the expression of IL26 mRNA in leprosy lesions is strongly differential, with higher expression in TT and RR lesions than LL lesions. Immunohistochemistry in lesions reflects the mRNA expression, with an abundance of IL-26 presence in PB lesions and a relative absence of IL-26 in MB lesions (40). Within the lesions, IL-26 colocalizes to the greatest extent with CD4+ T cells, presumably Th17 cells. Recombinant IL-26 has been demonstrated to have direct antimicrobial activity against M. leprae and M. tuberculosis in liquid culture. Furthermore, treatment of M. leprae or M. tuberculosis infected macrophages with IL-26 leads to a dose-dependent decrease in bacterial viability (Figure 4). Aside from direct antimicrobial activity against the bacteria, part of the antimicrobial ability of IL-26 is due to its ability to activate STING mediated autophagy, most likely through the formation of IL-26-DNA complexes (40). The unique role of Th17 cells to secrete the antimicrobial protein IL-26 provides these cells with additional tools to combat M. leprae in infected individuals.

**FIG 4** Direct antimicrobial pathways of T-cells in intracellular infection.

CD4+ T-cells secrete IL-26, a Th17 cytokine that has direct antimicrobial activity against the bacteria. Similarly, a subset of CD8+ T cell, termed tri-cytotoxic T-cell, armed with granzyme B, perforin, and granulysin is more effective in killing intracellular bacteria like M. leprae.

**CTL IN LEPROSY**

CD8+ T cells are activated in response to antigens presented on MHC class I, and secrete cytotoxic granule proteins like perforin, granzymes, and granulysin. Perforin forms pores in the membranes of target cells, while granzymes proteolytically cleave caspases within the cell, leading to apop-
tosis of the cells (119, 120). Granulysin is a pore-forming, lytic protein like perforin; however, it has also been shown to have broad antimicrobial activity against a variety of bacteria, including mycobacteria (121). Cytotoxic CD8⁺ T cells can also act indirectly through the secretion of IFN-γ, stimulating infected macrophages to kill intracellular pathogens (122).

Several lines of evidence support the hypothesis that CD8⁺ T cells play a protective role in host defense against mycobacteria. In animal models, adoptive transfer of CD8⁺ T cells protects against the development of active disease upon *M. tuberculosis* exposure, and the depletion of CD8⁺ T cells leads to an increased bacterial burden in the chronic phase of infection (123, 124, 125). In humans, antigen-specific cytotoxic CD8⁺ T cells, which can be isolated from the peripheral blood of patients with *M. tuberculosis* and *M. leprae*, are present in tuberculosis and leprosy granulomas, respectively (6, 126, 127, 128). Although CD8⁺ T cells predominate within LL lesions (129), they express a CD8⁺ T-suppressor phenotype (6) and secrete IL-4 (2, 7). In contrast, CD8⁺ T cells in TT lesions express a T-cytolytic phenotype (6), yet little is known about the antigens they recognize (130). Cytotoxic CD8⁺ T cells are able to kill mycobacteria-infected cells and decrease mycobacterial viability in a process mediated by the release of cytotoxic granule proteins (131). Mycobactericidal capacity is dependent upon granulysin; however, perforin is required for granulysin entry into the infected cell and antimicrobial activity (132). CD8⁺ T cells are cytolytic against cells pulsed with *M. leprae* antigens or infected with *M. leprae* (133, 134). These CD8⁺ T cells express perforin and the antimicrobial protein granulysin, mounting an antimicrobial response against *M. tuberculosis* (131, 132). Granulysin-expressing cells are more frequent in TT than LL lesions (135).

A subset of cytotoxic T cells, termed tri-cytotoxic cells due to their simultaneous expression of granzyme B, perforin, and granulysin, have a higher frequency in the blood of TT patients as compared to LL patients (136). Armed with three cytotoxic granule proteins, these cells are theoretically more potent at killing intracellular bacteria (Figure 4). Primary cells enriched for tri-cytotoxic CD8⁺ T cells have been shown to be more effective at killing *M. leprae* within infected macrophages than other CD8⁺ T cell subsets. The tri-cytotoxic T cells were demonstrated to express surface receptors typically associated with natural killer cells, including KLRC1 and KLRC2, which were functional in modulating cytotoxic granule release. This highly cytotoxic CD8⁺ T cell subset has yet to be demonstrated to be present within leprosy granulomas; however, its correlation with the resistant form of the disease and ability to kill intracellular *M. leprae* indicate its likely role in host defense against leprosy.

**Permissive Adaptive Immunity**

**TH2 RESPONSES IN LEPROSY**

Th2-type cytokines are associated with B-cell activation, heightened antibody production, and inhibition of several macrophage functions, which can counteract Th1-mediated antimicrobial
responses (11, 12). In the leprosy spectrum, LL patients were shown to exhibit high titers of antibodies against *M. leprae* components and an enrichment of B-cell genes expression in skin lesions when compared to TT lesions (137). The high levels of IL-5 detected in LL patients were shown to contribute to the humoral immune response observed in this pole due to this cytokine’s ability to increase IgM secretion levels synergistically with *M. leprae* in PBMCs (137).

The high levels of IL-4 identified in LL skin lesions can contribute to unresponsiveness to *M. leprae* by exerting suppressive effects in innate and adaptive immune cells. IL-4 is known to promote downregulation of TLR2 expression in monocytes and dendritic cells, as well as inhibit TNF-α and IL12 p40 release by monocytes stimulated with 19-kDa lipopeptide (60). Furthermore, IL-4 can decrease IL12 p40 release by monocytes stimulated with *M. leprae* alone (55) and can induce expansion of the CD8+ T cells populations found in LL patients, which have been shown to suppress CD4+ T cell responses (138).

**SUPPRESSOR CD8+ T CELLS**

In LL lesions, CD8+ T cells are the predominant type of T cell, with a CD4:CD8 ratio of 0.6 (Figure 5) (139). Most of the CD8+ T cells in LL lesions are CD28–, which is associated with a suppressive phenotype (6, 140). In agreement with this phenotype, CD8+ clones derived from leprosy lesions...
secretes large amounts of IL-4 and very little IFN-γ (7, 141). In response to *M. lepra* and *M. leprae* antigens, lesion-derived CD8⁺ suppressor cells do not proliferate and simultaneously limit the proliferation and cytokine secretion of bystander T cells (141). Thus, CD8⁺ suppressor cells induce anergy against *M. leprae*-specific T-cell responses (142, 143). Of note, these CD8⁺ suppressor T cells are part of a larger family of T cells with suppressive activity including regulatory T cells (Tregs), which are typically CD4⁺ and express the master transcriptional regulator FOXP3. However, CD8⁺CD25⁺FOXP3⁺ Tregs have been identified and are more frequent in the blood of LL patients as compared to TT patients, although these cells have not yet been detected within leprosy lesions (144, 145). Therefore, CD8⁺ T cells play a significant role in the suppression of CMI in states of MB leprosy, mediated by both CD8⁺CD28⁻ suppressor T cells and CD8⁺ Tregs.

**CD4⁺ REGULATORY T CELLS**

CD4⁺ Tregs modulate the immune response by suppressing effector T-cell functions to minimize inflammation and autoimmunity (146). Suppressor T cells in leprosy were found by cloning T cells from the skin of LL and borderline lepromatous patients (7, 141). These suppressor T cells (now called Tregs) were able to suppress the mycobacteria-specific T-cell response. Further studies characterized this population as CD4⁺CD25⁺ T cells expressing FoxP3 in humans (147). Tregs contribute to the dysfunction of *M. leprae*-specific T cells by secreting suppressive cytokines IL-10 and TGF-β, leading to the loss of T-cell functions. Several studies have reported an increase in the frequency of Tregs in the peripheral blood of LL and RR patients compared to TT patients and other clinical forms (144, 145, 148, 149, 150, 151, 152). In all clinical forms, Foxp3⁺ cells were present in epithelioid or macrophage infiltrates (153). This suggests a functional interaction between Tregs and macrophages, which has been shown by the presence of CD163⁺ macrophages in the vicinity of Foxp3⁺ cells in LL lesions (149, 153, 154). Similar to the polarized environment of Th1 vs Th2 in leprosy patients, IL-10⁺ Tregs negatively correlate with the presence of IL-17⁺ Th17 in LL patients. Tregs in LL patients also upregulate inhibitory receptors. Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1), and its ligand PD-L1 further contribute to T-cell exhaustion and anergy (148). Recently, a novel inducible Treg (iTr35) population that secretes the immunosuppressive cytokine IL-35 has been described (155). As a member of the IL-12 family, IL-35 is a dimeric protein with IL-12a and IL-27b chains encoded by IL12A and EBI3 genes, respectively (156). Similar to IL-10 and TGF-β, IL-35 suppresses T-cell proliferation and effector T-cell functions. The frequency of iTr35 is higher in LL and TT patients compared to healthy controls (156). Furthermore, IL-35 production positively correlates with the suppressed immune state and higher bacteriological index from borderline tuberculoid to LL. While it is clear Tregs play a role in the progression of leprosy, the mechanisms of its inhibitory function on Th17 cells and how Tregs’ functions change with the various clinical forms of leprosy are still unclear. Further investigation is necessary to understand immunological features that Tregs mediate and regulate in leprosy.
GAMMA DELTA (γδ) T CELLS

Unlike the T-cell subsets we have mentioned thus far, which express T-cell receptors (TCRs) composed of alpha and beta subunits, γδ T cells express TCRs composed of gamma and delta subunits. γδ T cells account for roughly 4% of the CD3+ cells in blood, lymphoid tissues, and skin (157). Unlike typical T cells, they seem to be more like innate cells, as evidenced by their expression of a restricted range of invariant T-cell receptors that recognize the phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate (HMBPP) as well as by their ability to be activated by cytokines in a nonspecific manner (158, 159). They have been shown to be potent producers of both IFN-γ and IL-17A (160, 161). γδ T cells have been extensively characterized in the setting of M. tuberculosis infection, and some evidence exists for a role for γδ T cells in M. leprae infection.

An early role for γδ T cells in the immune response to leprosy was established by our lab, finding that γδ T cells made up 25–35% of the CD3+ T cells within granulomas of patients in RR and in lepromin skin tests, compared to just 5% in lesions of other forms of leprosy (162). γδ T-cell lines isolated from the lesions proliferated in response to sonicated M. leprae. Furthermore, supernatants of M. leprae-stimulated γδ T cells induced aggregation of monocytes in culture, implicating γδ T cells in the process of granuloma formation. The γδ T cells within leprosy lesions consisted of both the Vδ1 and Vδ2 variety, with a Vδ1:Vδ2 ratio of 1:2 as compared to 1:9 in blood (162, 163). Both Vδ1 and Vδ2 were present within the dermis; however, only Vδ1 cells were present in epidermis. More recently, γδ T cells were demonstrated to be significantly enriched in the peripheral blood of patients undergoing RR and ENL reactions as compared to TT and LL (164). These cells produce both IFN-γ and IL-17, including the subset of γδ T cells that express FOXP3. Importantly, the γδ T cells from reactional states also produce less TGF-β than those from TT and LL patients, contributing to the overall proinflammatory state of the reaction. In contrast, it has also been shown that CD25+FOXP3+ γδ T cells are present in lesions of LL and are functionally immunosuppressive (165). However, in the context of the inflammatory environment of leprosy reactions, which have increased IL-23 and IL-1β, it is speculated that these previously regulatory FOXP3+ γδ T cells may become proinflammatory IL-17 and IFN-γ producers (164).

Humoral Immunity

B CELLS

While the main function attributed to B cells is antibody production, B cells can also act as professional APCs and can detect, process, and present antigens, leading to the activation of CD4+ and CD8+ T lymphocytes (166). Although B cells have been identified in leprosy tissue and polyclonal and specific anti-M. leprae antibodies have been demonstrated in the serum of leprosy patients, the role of B cells in the pathogenesis of leprosy is poorly understood.
The presence of B cells in leprosy tissue was initially described by Ridley (167). Recently, B cells have been detected through the entire spectrum of leprosy, not only in the lepromatous side of the disease. Granulomas from tuberculoid patients have shown a higher number of CD20 cells (immature and mature B cells), while biopsies from LL patients have shown more CD138+ cells (plasma cells). Plasma cells have been observed in borderline tuberculoid patients but in a lesser amount (137, 168, 169, 170).

The role of humoral immune response in defense against intracellular pathogens including \textit{M. leprae} is generally thought to be irrelevant. The antibody response to \textit{M. leprae}, as well as to specific antigens, in LL patients has been evaluated. In LL, there is a polyclonal activation of all isotypes (IgM, IgG, and IgA) and specific antibody responses to \textit{M. leprae} are detected in IgG1, IgG2, and IgG3 subclasses. Very little IgG4 or IgE has been detected in any group of leprosy patients.

Antibody responses to PGL-1 and its glycoconjugates have been shown to be present in 90–95% of LL patients and 25–60% of TT patients. Specific antibodies to PGL-1 are predominantly IgM (171, 172, 173, 174). Antibodies to the \textit{M. leprae}-specific antigen ‘Leprosy IDRI Diagnostic-1 with Natural Disaccharide with Octyl ligation’ (LID-NDO) are increased in MB patients (see Chapter 7.1). However, the role of these antibodies in the pathogenesis of leprosy is poorly understood. The correlation of antibodies with the progressive infection suggests that they play no role in protection, but some studies suggest an early role in leprosy and other mycobacterial Infections (175).

Analysis of gene expression profile data, obtained from LL and TT skin lesions using knowledge-guided bioinformatics analysis, has identified a number of B-cell-related genes that belong to the B-cell receptor signaling and the functional groups ‘proliferation of B lymphocytes’ and ‘quantity of B lymphocytes’ (176). Moreover, analysis of the category ‘physiological system development and function’ identified ‘Humoral Immune Response’ as the second highest biological function, suggesting a role for B cells and immunoglobulins in LL. In this study, a pathway analysis of the increased B-cell genes in LL revealed a potential network, linking the expression of IgM and interleukin-5 (IL-5). IL-5 was found to synergize \textit{in vitro} with \textit{M. leprae} to enhance total IgM secretion from PBMCs, suggesting a role for IL-5 in the increase of IgM production from B cells (137).

B cells may also be involved in disease pathology, especially in autoimmune disorders. In fact, studies of leprosy sera have identified a wide spectrum of autoantibodies such as anticardiolipin (aCL), antinuclear antibodies (ANA), rheumatoid factor, and antiphospholipid antibodies. Autoantibodies such as aCL have been reported to be raised in 37–98% of the patients with LL, providing a possible mechanism for the development of autoimmunity (177, 178). The production of antibodies at the site of disease may also contribute to immunopathology and tissue injury in leprosy. In fact, 30–50% of LL patients can develop reactions like ENL. The pathogenesis of ENL (see Chapter 2.2) is attributed to antibodies and immune complex deposition, as evidenced by granular deposits of immunoglobulin and complement in a perivascular and extravascular distribution, detection of immune complexes in vessel walls, and evidence of damaged endothelial cells (179).
A high bacillary index has been associated with high antibody levels and with the development of leprosy reactions and neuritis. High levels of anti-PGL-I antibodies at diagnosis or after treatment have been associated with a higher risk of developing leprosy reactions, especially ENL (180).

MB patients who subsequently developed ENL had increased levels of IgM, IgG1, and C3d at leprosy diagnosis compared to those who did not, and thus these serum markers could potentially be used to estimate the risk of developing reactions (181). In a recent study of ENL patients, activated memory B cells were increased in untreated patients with ENL reactions, suggesting a role for these cells in the ENL pathology. In this study, the percentage of total B cells in peripheral blood was not significantly different in LL patients as compared with ENL patients. However, after treatment, the proportion of B cells was significantly reduced from 9.5% to 5.7% in patients with ENL, suggesting that the depletion of B cells could be effective in the treatment of ENL. A decreased number of tissue-like memory B cells in untreated ENL patients compared to LL controls was also reported (182).

Additionally, increased levels of antibodies against LAM, a polysaccharide antigen present in *M. leprae*, have been associated with the development of RR (183). Antibodies against neural proteins such as S100, ceramides, and sphingolipids have been demonstrated in leprosy patients, suggesting a pathogenic role for antibodies in the development of nerve damage. However, it is unclear if any or all of these antibody levels have a predictive value in the early diagnosis of leprosy reactions.

IL-10-producing regulatory B cells (Bregs) are a small population of B cells that participate in the suppression of autoimmune disease. An elevated number of Bregs increases susceptibility to pathogens, prevent host defense against infection, and promote metastasis and tumor growth by converting CD4+ T cells into Tregs. IL-35 induces Bregs and promotes their conversion to IL-10-producing cells. IL-35-producing Tregs and Bregs have been found to be high in LL patients, suggesting that these IL-35-producing cells may be associated with the progression of the disease (156). In summary, the mechanisms by which B cells and humoral immunity regulate the immune response to *M. leprae* remain to be clearly defined.

Conclusion

The adaptive immune response is essential in controlling *M. leprae* infections and thereby influences the clinical manifestations of the disease. TT and LL patients represent two ends of a spectrum of immunity in leprosy (Figure 6). TT patients are characterized by a strong cell-mediated immune response while LL patients feature the activation of pathways that inhibit CMI and a high humoral response. The effective cell-mediated immune response involves the local production of Th1 cytokines, γδ T cells, CD1a-restricted T cells, Th17 cells, and CD8+ cytotoxic T cells. In contrast, in patients with progressive infection, Th2 cytokines, CD8+ suppressor T cells, CD4+ Tregs, and type I interferon responses predominate. While humoral responses are mounted in both TT and LL lep-
rosy, as presented above, the fact that the predominant immune response mounted in LL leprosy strongly inhibits CMI highlights the fundamental role that this type of adaptive immunity plays in effective immune responses to *M. leprae* infections. Taken together, these cell-cell interactions play an important part in influencing immune responses in leprosy.

FIG 6 Summary of adaptive immunity in leprosy.

Protective cell-mediated immune responses mediated by Th1 and Th17 cytokines and cytotoxic CD8⁺ T cells lead to fewer skin lesions and a lower bacillary load. In permissive forms of leprosy, Th2 cytokines, regulatory and suppressor cells, and humoral responses impair CMI, leading to numerous skin lesions and a higher bacillary load.

References


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