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The innate immune response in leprosy

Robert L Modlin^{1,2}

¹Division of Dermatology, Department of Medicine, David Geffen School of Medicine at University of California at Los Angeles (UCLA), CA 90095, USA

²Department of Microbiology, Immunology and Molecular Genetics, UCLA, David Geffen School of Medicine at University of California at Los Angeles, 90095, USA

Abstract

Investigation into the innate immune response in leprosy has provided insight into immunoregulation in human infectious disease. Key advances include the role of pattern recognition receptors in recognizing pathogen-associated molecular patterns of *Mycobacterium leprae*, cytokine release by innate immune cells, macrophage and dendritic cell differentiation, as well as antimicrobial effector pathways. These insights provide targets for therapeutic intervention in modulating the course of leprosy and other chronic infectious diseases.

Leprosy as a model

Leprosy, caused by the intracellular pathogen *Mycobacterium leprae*, offers an attractive model for investigating the regulation of immune responses to infection. First, the disease itself still poses a significant health and economic burden on developing countries [1]. Although multidrug therapy is reducing the number of active cases to approximately 0.5 million, the number of new cases per year is not declining. Furthermore, patients ‘cured’ of the infection, have permanent neurologic deficits, can relapse or subsequently undergo tissue-damaging reactions. Second, leprosy is a disease that presents as a spectrum, in which the clinical manifestations correlate with the type of immune response to the pathogen [2], providing an extraordinary opportunity to study resistance versus susceptibility to a microbial pathogen.

At one end of the disease spectrum, patients with tuberculoid leprosy (T-lep) typify the resistant response that restricts the growth of the pathogen. The number of lesions is few and bacilli rare, although tissue and nerve damage are frequent. At the opposite end of this spectrum, patients with lepromatous leprosy (L-lep) represent susceptibility to disseminated infection. Skin lesions are numerous and growth of the pathogen is unabated. These clinical presentations correlate with the level of cell-mediated immunity (CMI) against *M. leprae*. The standard measure of CMI to the pathogen is the Mitsuda reaction or lepromin skin test, a three-week response to intradermal challenge with *M. leprae*. The test is positive in T-lep patients but negative in lepromatous patients. Interestingly, antibody responses are greater in L-lep patients, indicating that humoral immunity does not contribute to host defense.

The inverse correlation between CMI and humoral immunity was initially investigated in terms of the adaptive immune response. Distinct T cell subsets correlate with the response to *M. leprae* including CD4 and CD8 T cells [3] and the T cell cytokine patterns they produce [4–6]. CD4+ T cells that produce the type 1 or Th1 cytokine pattern including IFN- γ predominate in T-lep lesions; whereas, CD8+ T cells that produce the type 2 or Th2 cytokine pattern

including IL-4 predominate in L-lep lesions. The nature of the adaptive T cell response is determined in part by the instruction of the innate immune response. Therefore, leprosy provides an excellent model to investigate mechanisms by which the innate immune system determines outcome in human infectious disease.

Recognition of *M. leprae* by the innate immune system

In 1884, Metchnikoff described the key features of the modern innate immune system. Cells that mediated the innate response, which he termed 'phagocytes', were able to rapidly recognize, phagocytose, and destroy the foreign invader [7•]. Over 100 hundred years later, we have gained considerable insight into some of the mechanisms by which the innate immune system recognizes microbial invaders. Cells of the innate immune system are equipped with germ line encoded pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs), which are shared among groups of pathogens.

Toll-like receptor 2/1 (TLR2/1)

From the study of *M. tuberculosis* and related species, we have gained insight into the receptors that mediate the response to mycobacteria. Several Toll-like receptors (TLRs) clearly mediate innate immune recognition of *M. tuberculosis* and related species. Experiments performed in the Modlin lab [8] and other labs [9] led to the exciting finding that microbial lipoproteins trigger host responses via TLR2, requiring the acyl functions for activity. Subsequently, triacylated lipoproteins were found to activate TLR2/1 heterodimers [10], whereas diacylated lipoproteins were found to activate TLR2/6 heterodimers. TLR2 also mediates the response to the lipoarabinomannan from rapidly growing mycobacteria [11], and in combination with MARCO and CD14 to mycobacterial trehalose dimycolate [12•]. TLR2 has been shown to be important for resistance to TB in mouse models of infection [13–15].

The expression and activation of TLRs was investigated in leprosy [16]. TLR2–TLR1 heterodimers mediated cell activation by killing *M. leprae*, indicating the presence of triacylated lipoproteins. A genome-wide scan of *M. leprae* detected 31 putative lipoproteins. Synthetic lipopeptides representing the 19-kDa and 33-kDa lipoproteins activated both monocytes and dendritic cells (DCs). Additional studies demonstrated that the *M. leprae* 33-kDa lipoprotein [17] and the *M. leprae* major membrane protein-II (MMP-II) [18], also a lipoprotein, triggered TLR2 responses, requiring the acyl functions and the polypeptide region for optimal activity. Activation was enhanced by type 1 cytokines and inhibited by type 2 cytokines. In addition, the type 1 cytokines, IFN- γ and GM-CSF, enhanced TLR1 expression in monocytes and DCs, respectively, whereas the type 2 cytokine, IL-4 downregulated TLR2 expression. TLR2 and TLR1 were more strongly expressed in lesions from the localized T-lep form as compared with the disseminated L-lep form of the disease.

Ultimately, the activation of the TLR2/1 heterodimer by *M. leprae* lipopeptides triggered the production of cytokines such as TNF- α , as part of the acute inflammatory response [16] and IL-12, which mediates the instructive role of the innate immune response in instructing the adaptive type 1 or Th1 cytokine response [16]. Activation of TLR2/1 also upregulated receptor–ligand pairs as part of an autocrine response including GM-CSF and its receptor leading to the expansion of DC and IL-15 and its receptor components leading to M Φ differentiation [19]. Together, these data provided evidence that regulated expression and activation of TLRs at the site of disease contribute to the host defense against microbial pathogens.

A number of mechanisms have been identified, which regulate TLR function in leprosy. In addition to the ability of IL-4 to downregulate TLR2/1 expression, it also inhibits TLR2/1-induced cytokine responses [16]. IL-10 had no effect on TLR2/1 expression but strongly inhibit TLR2/1-induced cytokine release [16]. Activation of LILRA2, which is differentially

expressed in L-lep versus T-lep lesions, inhibited TLR2/1-induced IL-12 release but preserved IL-10 release [20]. Similarly, oxidized phospholipids inhibited TLR2/1-induced IL-12 release but preserved IL-10 release [21•]. The ability of *M. leprae* to upregulate tryptophan aspartate-containing coat protein (TACO), known to be expressed in macrophages containing *M. leprae* *in vitro* and in disease lesions [22], was shown to downregulate TLR2-mediated signaling [23••].

TLR2 and TLR1 SNPs

Polymorphisms in TLR2 and TLR1 genes have been investigated in patients with leprosy. Initially, a TLR2 SNP was found to be associated with susceptibility in leprosy [24], prompting functional investigations using transfections of the altered TLR2 gene [25–28]. This SNP has been recently identified in a pseudogene and does not encode a TLR2 transcripts [29]. However, there is compelling data to suggest that polymorphisms in the TLR1 gene may contribute to the TLR2/1 response to lipopeptides, and to the pathogenesis of leprosy. The TLR1 T1805G SNP (I602S), rs5743618 affects TLR1 surface expression and subsequent activation by mycobacterial lipopeptides [30••,31••]. The TLR1 I602S SNP was associated with a decreased incidence of leprosy [30••] and protection against reversal reaction [31••]. The TLR1 A743G SNP (N248S) rs5433095 is in linkage disequilibrium with TLR1 1805, but had little effect on function in transfection studies when separated from the TLR1 1805 allele [32]. TLR1 248SS is associated with protection against leprosy [33]. It would seem puzzling that a polymorphism that would reduce TLR2/1 signaling would protect against leprosy and reversal reaction. However, Johnson *et al.* reason that the TLR response may be critical during the acute infection, but a moderation of the innate response may be beneficial in chronic infectious diseases such as leprosy [30••]. Consistent with this hypothesis is the finding that TLR2/1 activation can lead to tissue injury, including nerve damage in leprosy [34].

Other TLRs and PRRs

TLR4 has been reported to be required for the host defense against *M. tuberculosis* [35], and shown to mediate the response to mycobacterial heat shock proteins [36], although these studies are frequently criticized because the recombinant proteins used may contain amounts of endotoxin sufficient to activate TLR4 [37,38]. Two TLR4 SNPs, TLR4 D299G and T399I were found to be associated with a protective effect against leprosy [39••]. Finally, TLR 9 has a role in recognition of bacterial CpG DNA, participating in the response to mycobacteria [15]. A polymorphism in TIRAP, a signaling molecule downstream of the TLRs, TIRAP S180L has been associated with protection against leprosy infection [40].

A comprehensive study of TLR pathway gene polymorphisms in mycobacterial infection analyzed 149 SNPs in 18 genes involved in the TLR pathway in susceptibility to TB [41••]. Although SNPs in the TLR9 and TOLLIP genes were found to be associated with TB, the allele frequencies were low. However, four polymorphisms, all in the TLR8 gene showed statistical evidence of association with resistance to TB (odds ratio 1.2–1.8) and were prevalent (ranging from 25 to 75%) in various populations. These results warrant studying whether TLR8 polymorphisms play a role in protection against leprosy infection.

Nucleotide-binding oligomerization domain 2 (NOD2) is a cytoplasmic receptor belonging to the NOD-like receptor family. It not only shares homology to TLRs in containing leucine rich repeats but also encodes a CARD domain. It mediates the response to peptidoglycan, including that derived from mycobacteria, by sensing muramyl dipeptide (MDP) [42,43]. Triggering of NOD2 by MDP activates NF- κ B through the adaptor molecule, RIP2. In addition, MDP can also activate the inflammasome, by recruitment of caspase-1, leading to the proteolytic cleavage and activation of IL-1 β [44]. NOD2 deficiency in a mouse model of TB resulted in increased susceptibility to infection at late timepoints [45,46]. NOD2 polymorphisms, not those

associated with Crohn's disease, were shown to be associated with susceptibility to TB [47]. NOD2 was not only found to mediate the response to TB in human monocytes and synergize with lipoprotein in activating monocyte cytokine responses [48], but also synergized with transfected DNA in activating the IFN- β pathway [49].

Vitamin D and innate immunity

The ability of TLRs to trigger a direct antimicrobial activity is a major aspect of their role in innate immunity. In mouse monocytes, TLR2-induced antimicrobial activity is NO dependent, but in human monocytes is NO independent [50]. Instead, a key antimicrobial mechanism for TLR-activated human monocytes involves induction of the 25-hydroxyvitamin D3-1 α -hydroxylase (CYP27b1), which converts the 25D into the active 1,25D form, upregulation and activation of the vitamin D receptor (VDR) and downstream induction of the antimicrobial peptide cathelicidin [51,52,53–55]. The ability of TLR2/1 activation to upregulate expression of CYP27b1 and the VDR is IL-15 dependent [19]. Simultaneous triggering of IL-1 β activity and activation of the VDR induces DEFB4, also required for antimicrobial activity [56]. The TLR-induction of antimicrobial peptides have been preserved during evolution; activation of drosophila Toll, which has been shown to trigger induction of several antimicrobial peptides [57]. An important aspect of this study was the discovery that induction of cathelicidin required the use of 25D-sufficient human serum and was not observed with 25D-insufficient human serum or fetal calf serum (which is also 25D-insufficient).

There is evidence to suggest that the vitamin D antimicrobial pathway may contribute to disease outcome in leprosy. Several studies suggest that polymorphisms in the VDR are associated with the type of disease, specifically the Taq I polymorphism [58–60]. Analysis of gene expression profiles in leprosy lesions indicated that genes encoding for key components of the vitamin D antimicrobial pathway were differentially expressed in T-lep versus L-lep lesions [61]. The induction of antimicrobial peptides is thought to be relevant to host defense in leprosy, given the finding that a polymorphism in the NF- κ B1 putative binding site of DEFB1 (human β -defensin 1), DEFB1 G668C is associated with L-lep disease [62].

Macrophages in L-lep lesions accumulated host-derived oxidized lipid

In Metchnikoff's model of innate immunity, recognition of the microbial pathogen was followed by phagocytosis. A key cell of the mammalian innate immune system that mediates phagocytosis of microbial pathogens is the macrophage (M Φ). By conventional histology, the M Φ in the different forms of leprosy are distinct. In T-lep lesions, the M Φ is thought to be activated, resembling epithelial cells, and are hence called epithelioid cells. In L-lep lesions, the M Φ besides containing numerous bacilli have a characteristic foamy appearance, first described by Rudolf Virchow in 1863. 'In the fresh state they have one characteristic that is especially noteworthy, i.e., their tendency to form a sort of vacuole, apparently from taking up water, so that under the circumstances they acquire a wholly physaliferous appearance' [63]. It has generally been thought that such cells, termed Virchow cells, lepra cells or foam cells are M Φ containing large amounts of lipids including phospholipids and fatty acids, presumed to be of mycobacterial origin [64].

By probing the profiles of differentially expressed genes in T-lep and L-lep lesions [20], we found a marked upregulation of host lipid metabolism genes in the L-lep versus T-lep lesions [21]. Moreover, the lipid within foam cells in L-lep lesions contains host-derived oxidized phospholipids. *In vitro*, we found that mycobacteria induce in M Φ the intracellular accumulation of a specific host-derived oxidized phospholipid, 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphorylcholine (PEIPC) providing a mechanistic link to the foam cells found in atherosclerosis lesions. In atherosclerosis, foam cells are derived from monocytes that enter the subendothelial space, differentiate into M Φ , and endocytose

modified forms of LDL [65]. They are defined by the intracellular presence of distinct components of oxLDL: oxidized phospholipids, esterified cholesterol, and apolipoprotein B (ApoB); the latter is also expressed in L-lep lesions [66]. Foamy, lipid-laden MΦ are a frequent pathological observation in tuberculosis [67–72], with mycobacterial components such as mycolic acid able to promote MΦ accumulation of cholesterol ester and lipid body formation [73–76]. The ability of mycobacteria to promote lipid body formation appears to be dependent on TLR2 signaling [76] and PPAR-γ activation [77]. Furthermore, the accumulation of lipids in phagosomes in L-lep lesions involves lipid-droplet associated proteins adipose differentiation-related protein (ADRP) and perilipin [78••].

Presumably, the accumulation of host-derived lipids favor mycobacterial growth, which *in vivo* depends on the use of fatty acids by the glyoxylate shunt [21•]. The metabolism of host fatty acids to methyl malonyl CoA is coupled with *M. tuberculosis* pathogenesis through its incorporation into lipid-containing virulence factors [21•]. In addition, mycobacterial infection and host-derived oxidized phospholipids both inhibited innate immune responses, and this inhibition was reversed by the addition of normal HDL, a scavenger of oxidized phospholipids, but not by HDL from L-lep patients. These data point to striking similarities in the pathogenesis of foam cell formation in mycobacterial infection to atherosclerosis, implying a common metabolic and inflammatory process.

Divergence of macrophage phagocytic and antimicrobial programs in leprosy

In addition to their phagocytic function, MΦ also mediate an antimicrobial activity against infectious agents. The mechanisms that regulate these MΦ antimicrobial and phagocytic functions are central to our understanding of innate immune responses against microbial pathogens. Despite the histologic differences of MΦ infiltrating T-lep versus L-lep lesions, the frequency of CD209 expressing MΦ is similar in the lesions of all forms of the disease [19].

CD209 is a C-type lectin that contains carbohydraterecognition domains (CRD) enabling cytokine-derived DCs to recognize pathogens containing mannose-rich glycoconjugates, including the mycobacterial lipoglycan mannosylated lipoarabinomannan (ManLAM) [79]. This recognition allows CD209 to mediate binding and phagocytosis of a broad range of pathogens, including, *M. leprae* [80] and is expressed in both the T-lep and L-lep forms of leprosy [19].

Although MΦ in both T-lep and L-lep lesions expressed CD209 [19], we reasoned that different MΦ functional programs contributed to the pathogenesis of leprosy. Clearly, the distinct T cell cytokine patterns in leprosy lesions can influence MΦ function [5,81]; however, we thought it was likely that the cytokine of the innate immune response had direct effects on MΦ function. Of the innate immune cytokines known to regulate MΦ function, T-lep lesions express IL-15 [5,81], whereas, L-lep lesions are characterized by the expression of IL-10 [5,81], prompting the comparison of the IL-15 and IL-10 induced MΦ differentiation [61•]. Both IL-10 and IL-15 upregulated CD209 expression on monocytes, however they induced MΦ functional programs. IL-10 induced the phagocytic pathway, including a scavenger receptor program, resulting in the phagocytosis of mycobacteria and oxLDL. In contrast, IL-15 induced the vitamin D-dependent antimicrobial pathway, yet the cells were less phagocytic. The differential regulation of macrophage functional programs was confirmed by analysis of the spectrum of leprosy lesions: the macrophage phagocytosis pathway was prominent in the clinically progressive L-lep form, whereas the vitamin D-dependent antimicrobial pathway predominated in the self-limited T-lep form of the disease and in patients undergoing reversal reactions from the multi-bacillary to the self-limited form. Polymorphisms in the IL-10 locus have been investigated by several groups with conflicting results [60,82,83•,84–86]. Paradoxically, one IL-10 promoter

polymorphism, 819C/T, was associated with susceptibility to leprosy, and could lead to decreased IL-10 production [82,83•,84]. In summary, these data indicate that macrophage programs for phagocytosis and antimicrobial responses are distinct and differentially regulated in innate immunity in bacterial infections.

Dendritic cell deficit in lepromatous leprosy

The ability of the innate immune system to instruct the adaptive T cell response is part of an effective host defense against intracellular pathogens. This instructive role of the innate immune system is primarily mediated by DCs, professional antigen-presenting cells [87] that are highly efficient in activation of T cell responses that provide CMI against the pathogen [88]. L-lep lesions are characterized by a marked deficit in DCs, both in the dermis and epidermis [89–91], a potential mechanism for the reduced cell-mediated immune responses in these lesions [2]. DCs differentiate from myeloid precursors, a process that can be interrupted by inhibitory receptors as well as host-derived oxidized phospholipids that are found in L-lep lesions [20,92]. In addition, peripheral monocytes from L-lep patients do not differentiate into CD1+ DC following TLR activation [19]. Expression of the costimulatory protein, B7.1, is also decreased in L-lep lesions [93], further suggesting impaired CMI. The activation and maturation of DCs can be directly inhibited by the pathogen itself, in contrast to other mycobacteria [94•], suggesting that the pathogen specifically subverts the generation of functional antigen-presenting cells. Together these studies suggest that *M. leprae* contributes to reduced cell-mediated immune responses in leprosy by disrupting the differentiation of DCs and their antigen-presenting capacity.

In conclusion, leprosy has provided an exciting model to investigate the key role of the human innate immune system in host defense against versus susceptibility to microbial infection. It is hoped that these insights will lead to new therapeutic interventions for leprosy and other infectious diseases worldwide.

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