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**Mycobacterium leprae** Inhibits Dendritic Cell Activation and Maturation

Rose Ann Murray,* Mahveen Ruby Siddiqui,† Megan Mendillo,‡ James Krahenbuhl,§ and Gilla Kaplan2*

Leprosy presents with a clinical spectrum of skin lesions that span from strong Th1-mediated cellular immunity and control of bacillary growth at one pole to poor Ag-specific T cell immunity with extensive bacillary load and Th2 cytokine-expressing lesions at the other. To understand how the immune response to **Mycobacterium leprae** is regulated, human dendritic cells (DC), potent inducers of adaptive immune responses, exposed to **M. leprae**, **Mycobacterium tuberculosis** (Mt), and **Mycobacterium bovis** bacillus Calmette-Guerin (BCG) were studied for their ability to be activated and to prime T cell proliferation. In contrast with Mt and BCG, **M. leprae** did not induce DC activation/maturation as measured by the expression of selected surface markers and proinflammatory cytokine production. In MLR, T cells did not proliferate in response to **M. leprae**-stimulated DC. Interestingly, **M. leprae**-exposed MLR cells secreted increased Th2 cytokines as well as similar Th1 cytokine levels as compared with Mt- and BCG-exposed cells. Gene expression analysis revealed a reduction in levels of mRNA of DC activation and maturation markers following exposure to **M. leprae**. Our data suggest that **M. leprae** does not induce and probably suppresses in vitro DC maturation/activation, whereas Mt and BCG are stimulatory. *The Journal of Immunology*, 2007, 178: 338–344.

Leprosy is a debilitating, chronic, infectious disease caused by **Mycobacterium leprae** that involves skin and peripheral nerves. Official reports received from 115 countries and territories place the prevalence of leprosy at the beginning of 2006 as 219,826 cases. Although there has been a dramatic decrease in new cases worldwide, there are still pockets of high endemicity (1). Clinical manifestations of the disease present as a spectrum in which protective cellular immunity correlates inversely with the bacillary load. At one pole is tuberculoid leprosy (T-lep), characterized by strong Th1 immunity and few bacteria in skin lesions; at the other extreme is lepromatous leprosy (L-lep), characterized by a high number of bacilli disseminated throughout the skin with lesions expressing high Th2 type cytokines and with poor systemic Ag-specific Th1 cellular immunity (2).

Naive T cells require two different signals from APCs to become activated. Signal 1 depends on the TCR-recognizing Ag presented by MHC molecules located on the surface of the APC. Signal 2 depends on the production of cytokines and their expression on the surface of the APCs of costimulatory molecules that interact with T cell ligands. The delivery of both signal 1 and signal 2 results in the activation of Ag-specific T cells (3). Signal 1, in the absence of signal 2, induces T cell anergy, and the absence of complete APC activation has been proposed to initiate a Th2 type response (4). Many microbial factors induce APC activation, resulting in cytokine i.e., TNF-α and IL-12, secretion and up-regulation of costimulatory molecules; together, these promote the development of Th1 cells. The early APC-mediated activation of naive T cells by **M. leprae** might result in Th1 immunity (T-lep) or in Th2-mediated anergy (L-lep), depending on the strength of the cytokine and the costimulatory signals accompanying TCR engagement.

Dendritic cells (DCs) are APC unique in their ability to induce primary T cell activation. This ability, however, depends on the state of DC maturation. Immature DCs (iDC) are highly phagocytic. Upon stimulation of iDCs with microbial products, the iDC surface-expressed TLRs trigger NF-κB and MAPK-mediated DC maturation and activation. Mature DCs become efficient APCs by increasing peptide loading and delivery of MHC molecules to the cell surface. In addition, expression of the costimulatory molecules CD86/B7.2, CD80/B7.1, CD83, and CD40 is up-regulated. Mature DCs migrate from the infected/inflamed tissue and enter lymphatic circulation, presumably directed by up-regulation of surface expression of CCR7, the only chemokine receptor expressed by mature DCs (5). **M. leprae** activates DCs via TLR2-TLR1 heterodimers, presumably through triacylated lipopolysaccharides the like 19-kDa lipoprotein (6). TLR2 polymorphisms have been linked to increased susceptibility to leprosy (7, 8).

Activation of an appropriate immune response is critical for maintaining well-regulated immunologic homeostasis. For example, chronically activated DCs have been proposed to mediate the presentation of self-nucleoprotein complexes in lupus (9). We hypothesized that **M. leprae** might subvert the activation of DCs, impacting on the development of normal protective Th1 type immunity. In the present report, we studied the effect of **M. leprae** on iDC activation and maturation and on DC-mediated primary T

*Abbreviations used in this paper: T-lep, tuberculoid leprosy; 7-AAD, 7-aminoactinomycin D; BCG, bacillus Calmette-Guerin; CBA, cytometric bead array; DC, dendritic cell; DC-SIGN, DC-specific ICAM-3-grabbing nonintegrin; iDC, immature DC; L-lep, lepromatous leprosy; LAM, lipooligosaccharidinemannnan; maturation (“cocktail”) mixture; MFI, mean fluorescence intensity; MOI, multiplicity of infection; Mtb, *Mycobacterium tuberculosis*; PGL-1, phenolic glycolipid 1.

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cell activation. We compared *M. leprae* to *Mycobacterium tuberculosis* (Mt) and *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) for their abilities to induce up-regulation of maturation markers, stimulate cytokine production, drive *T* cell activation, and induce the expression of genes associated with the phenotypic and functional maturation of DCs. We show that, relative to Mt and BCG, *M. leprae* does not induce DC maturation but in fact reduces the expression of various activation pathway genes. Interestingly, *M. leprae*-infected DCs seem to promote both a Th1 and a Th2 cytokine response during a MLR. Thus, early interactions between *M. leprae* and DCs could be important in determining whether *T* cell anergy or activation is achieved.

Materials and Methods

**Mycobacteria**

The *M. leprae* Thai-53 strain was used for these experiments. The mycobacteria had been maintained in programmed serial passage in the footpads of athymic *nude* mice infected with 5 × 10⁶ freshly harvested *M. leprae*. Briefly, bacilli were harvested from the footpads 3–4 mo after infection (at mid-log growth) as described previously (10). Bacilli were washed in Middlebrook 7H12 medium and enumerated by direct count according to Shepard’s method (11). The relative viability of *M. leprae* in the suspension was evaluated by using the LIVE/DEAD BacLight bacterial viability kit (Molecular Probes) as recently adapted for *M. leprae* (10). Pure preparations of bacilli free of mouse footpad tissue were obtained by treating the footpad suspension with 0.1 M NaOH for 5 min followed by neutralization with 0.1 M HCl and three washes with PBS. *M. leprae* were irradiated by gamma irradiation with 10⁴ grays from a cobalt source (12) and boiled for 10 min in water before use.

*M. bovis* BCG Pasteur strain (Trudeau Mycobacterial Culture Collection no. 1011) and *M. tuberculosis* H37Rv Pasteur strain (Trudeau Mycobacterial Culture Collection no. 102) (Mt) were grown in Middlebrook 7H9 medium (Difco) and kept as frozen stocks until they were used. Stocks were thawed, sonicated, and appropriately diluted for infection. For heat-killed mycobacteria, sonicated stocks were boiled in water for 10 min. All mycobacteria were passed through a 1-ml syringe attached to a 26gauge needle 3–4 times before preparing appropriate dilutions for infection. Unless otherwise stated, mycobacteria were added to leukocytes in culture at a multiplicity of infection (MOI) of five bacilli per cell.

**Generation of immature human DCs in vitro**

PBMCs were isolated from leukocyte preparations obtained at the New Jersey Blood Center (East Orange, NJ) by density-gradient centrifugation. Monocytes were purified by positive selection using magnetic bead-conjugated anti-CD14 Abs (Miltenyi Biotec). Monocytes were cultured in human serum (Gemcell) in RPMI 1640 (Invitrogen Life Technologies) and 10 mM HEPES (Invitrogen Life Technologies) with IL-4 (1 ng/ml) (Endogen) and GM-CSF (0.2 ng/ml) (Endogen) in 6-well plates (Falcon; BD Biosciences) at 3 × 10⁵/well at 37°C in 5% CO₂. IL-4 and GM-CSF were replenished on days 2 and 4. On day 6, nonadherent cells were harvested, cultured in 10% human serum in RPMI 1640 (R10) with IL-4 and GM-CSF, and plated in 24-well plates (Falcon; BD Biosciences) at 2.5 × 10⁴/well (for phenotyping and to generate target cells for allogeneic reactions) or in a 6-well plate at 2.5 × 10⁶/well (for RNA extraction).

**DC maturation in culture**

On day 6 of cell culture as described above, iDCs were either treated with medium (R10 plus IL-4/GM-CSF), medium plus a maturation mixture (designated hereafter as MC for maturation “cocktail”) consisting of TNF-α (10 ng/ml) (Alexis), IL-1β (10 ng/ml) (BD Pharmingen), and PGE₂ (1 mg/ml) (Sigma-Aldrich), medium plus mycobacteria, or medium plus mycobacteria plus MC. Cells were cultured under these conditions for 10 min in water before use. Staining was stopped with serum, followed by washing. Stained bacteria were incubated with iDCs at different ratios (0.01 and 0.1 DC:T cell) with DCs that had been treated on the GEArray membranes (SuperArray). The arrays were developed with x-ray film. The ScanArray 2 program (SuperArray) was used for image analysis and the GEArrayAnalyzer (SuperArray) for processing the raw data. Cells from different donors were used for each experiment. The signal from the expression of each gene was normalized to the signal derived from β-actin. A 2-fold difference in mRNA expression relative to DC treated with medium plus IL-4/GM-CSF was considered significant.

**Evaluation of phenotypic DC maturation**

The DC phenotype was assessed by flow cytometric analysis of surface marker expression. On days 6 and 8 of DC culture, cells were harvested and stained in 1% FCS (Gemcell) in PBS (Invitrogen Life Technologies) with fluorescently labeled Abs to CD14, CD25, CD40, CD80, CD83, CD86, CCR-7, HLA-DR, and HLA-ABC (BD Biosciences). Cell death was determined by staining with 7-aminoactinomycin (7-AAD) (BD Biosciences) and dead cells were excluded from analysis. Day 6 and day 8 geometric mean fluorescence intensity (MFI) for each marker was determined by flow cytometry on a FACScalibur (BD Biosciences) with analysis conducted in CellQuest software (BD Biosciences) and FlowJo software (Tree Star). Day 6 MFI was normalized to 100%, and day 8 MFI was then expressed as a percentage of day 6 MFI.

**Evaluation of *M. leprae*-DC binding**

*M. leprae*-iDC binding was evaluated by flow cytometric analysis. *M. leprae* was stained with PKH26 (Sigma-Aldrich) at 4 μM at room temperature. Staining was stopped with serum, followed by washing. Stained bacteria were incubated with iDCs at different MOI values (1, 5, 10, 40, 100 for *M. leprae*-DC) over the course of 4 h. The FL2 MFI was measured at the different time points for the different MOI values.

**Mixed lymphocyte reaction**

CD3⁺ cells were isolated from PBMCs with magnetic beads (Miltenyi Biotec). Cells were stained with CFSE (Molecular Probes) at 5 μM for 10 min at 37°C, the staining was stopped with R10, and the cells were incubated for 10 min at room temperature. Lymphocytes were then incubated at different ratios (0.01 and 0.1 DC:T cell) with DCs that had been treated for 2 days with medium plus either IL-4/GM-CSF (iDC) plus MC or MC alone (MOI of 5) or Mt (MOI of 5) or *M. leprae* (MOI of 5). Coculture went on for 6 days, when cells were harvested, fixed with FACS lysing buffer (BD Biosciences), stained with fluorescently conjugated Abs against CD4 and CD8 (BD Biosciences), and analyzed by flow cytometry on a FACScalibur (BD Biosciences).

**Cytokines**

Day 8 DC culture supernatants were harvested and used to evaluate cytokine production with commercial ELISA kits for IL-10, IL-12, and TNF-α (Endogen). Following 6 days of DC and T cell coculture (MLR), supernatants were collected from the DC:T cell 0.1 ratio and analyzed with human Th1/Th2 cytokine BD Biosciences cytometric bead array (CBA) kits. Flow cytometric acquisition was done on a FACScalibur (BD Biosciences), and further analysis was done with BD Biosciences CBA software.

**Statistics**

A nonparametric Mann-Whitney *t* test was used to compare means from two different groups. Two-way ANOVA was used to compare differences between groups for the MLR. A value of *p* < 0.05 was considered statistically significant.

**Results**

**Effect of cytokines on DC surface marker expression**

In vitro cultured human monocytes exposed for >6 days to IL-4 and GM-CSF developed into iDCs. Following the exposure of 6-day iDCs to IL-4/GM-CSF-containing medium for an extra 48 h, the geometric MFI of surface markers of maturation and activation (CD25, CD40, CD80, CD83, and CCR7) and MHC molecules (HLA-DR and HLA-ABC) among live cells (7-AAD⁻) was determined by flow cytometry (Fig. 1A). The MFI for these markers varied from ~12 to ~340. Addition of MC (IL-1β, PGE₂, and TNF-α) to the IL-4/GM-CSF-containing medium from day 6 to day 8 of culture resulted in further up-regulation of various markers (Fig. 1B). This effect was clearly MC dose dependent as shown by with A CDP-Star chemiluminescence substrate (PerkinElmer) and recorded with x-ray film. The ScanArray 2 program (SuperArray) was used for image analysis and the GEArrayAnalyzer (SuperArray) for processing the raw data. Cells from different donors were used for each experiment. The signal from the expression of each gene was normalized to the signal derived from β-actin. A 2-fold difference in mRNA expression relative to DC treated with medium plus IL-4/GM-CSF was considered significant.
the response of cells to titration of MC (Fig. 1, C and D). The results suggest that the assay selected is a sensitive method to compare the effect of different stimuli on iDC maturation.

**Effect of Mtb or BCG on iDC maturation**

We tested the activity of Mtb and BCG in the iDC maturation assay using viable or heat-killed (boiled) bacteria. Monocyte-derived iDCs were infected on day 6 at a MOI of 5 with live or heat-killed BCG or Mtb (Fig. 2). At this MOI the bacilli associated very efficiently with the iDC; all cells had at least one bacterium attached to them as determined by flow cytometric analysis of iDC exposed to PKH26 stained bacilli (data not shown). Both BCG and Mtb infection resulted in similar up-regulation of iDC surface markers of activation and maturation. Phenotypic iDC maturation appeared independent of mycobacterial viability. We therefore used heat-killed mycobacteria for subsequent experiments. In general, the extent of induction of phenotypic maturation by BCG and Mtb was somewhat lower than the response of the cells to MC.

**Effect of M. leprae on iDC maturation**

DC culture is conducted at 37°C; however, *M. leprae* viability is reduced at this temperature. We explored the possibility of maturing DC and/or activating iDC at 30°C to use viable *M. leprae*. A...
MOI values of \( M. \text{leprae} \) incubation.

MOI of 40 for \( F \) shown. At the time of bacterial addition (time 40 (\( F \)). MFI values of iDC without \( M. \text{leprae} \), and 100 (\( /H11005 \)) were clearly stimulatory (Fig. 3  under the same conditions and in the same donors, BCG and Mtb not up-regulate iDC surface markers of maturation. In contrast, to \( M. \text{leprae} \) exposed to iDC maturation, day 6 iDCs from the same blood donors were clearly stimulatory (Fig. 3B). This lack of response was noted even when MC was neither enhanced nor inhibited MC-induced iDC maturation (Fig. 3B). This lack of response was noted even when MC was titrated down to 0.5% (data not shown).

M. leprae binding and/or phagocytosis by iDC

To confirm that the lack of stimulation of surface marker expression was not a result of lack of bacillary association with APCs, we next examined the interaction of \( M. \text{leprae} \) with the iDCs in culture. iDC-M. leprae association and iDC viability following \( M. \text{leprae} \) infection at different MOI values (1, 5, 10, 40, 100 for \( M. \text{leprae}-\text{DC} \)) were evaluated over time (Fig. 4). By using fluorescently labeled \( M. \text{leprae} \), we determined mycobacteria-iDC association through changes in MFI. Over the course of 4 h, increasing numbers of bacteria were found to be associated with iDC. As MOI values increased, so did bacteria-cell association (Fig. 4A). We determined iDC viability by enumerating the percentage of cells that were stained with 7-AAD at different MOI values and found that up to a MOI of 40, DC viability was not diminished by interaction with \( M. \text{leprae} \) (Fig. 4F). Consequently, a MOI value of 5 or 10 was determined to be optimal for all experiments. At these

MOI values the bacilli were associated with the iDCs without inducing significant host cell death. In addition, an MOI of 5 was determined to induce optimal iDC stimulation by Mtb and BCG.

Effect of Mtb, BCG, or \( M. \text{leprae} \) on iDC secretion of Th1-type cytokines

The effect of exposure of iDC for 48 h to \( M. \text{leprae} \), BCG, or Mtb on cytokine production was evaluated. Both Mtb and BCG induced
iDC secretion of IL-12 and TNF-α (Fig. 5), with Mtb inducing higher TNF-α secretion despite inducing similar or often lower surface expression of maturation markers (Fig. 2). In contrast, M. leprae did not induce DC production of IL-12 or TNF-α, and its secretion profile was similar to that for iDCs. None of the three mycobacterial strains or MC induced significant secretion of IL-10 by iDCs.

**Effect of BCG, Mtb or M. leprae on functional iDC maturation**

We next determined whether the differences in iDC phenotypic maturation and activation affected T cell priming. Day 6 iDCs were treated with BCG, Mtb, or *M. leprae* for 48 h, after which they were irradiated and used as targets in a MLR (Fig. 6). *M. leprae*-infected DCs did not induce allogeneic lymphocyte proliferation at the 0.01 or 0.1 DC:T cell ratio; proliferation levels were actually lower than those observed in unstimulated iDC at these E:T ratios. In contrast, Mtb and BCG induced somewhat increased T cell proliferation compared with unstimulated iDCs. MC induced the highest proliferation of both CD4⁺ and CD8⁺ T cells (Fig. 6A). The cytokine profile in culture supernatants of the MLRs was evaluated (Fig. 6B). Surprisingly, although the Th2 cytokine (IL-4 and IL-5) response to *M. leprae* was higher than the response to Mtb or BCG, the Th1 cytokine (IFN-γ and TNF-α) response to *M. leprae* was similar to the response induced by the other mycobacteria.

**Effect of *M. leprae* on DC expression of activation pathway genes**

RNA was isolated from DCs treated with medium (iDC), Mtb, BCG, or *M. leprae*, and cDNA was synthesized from RNA and used to probe a DC-specific array. We compared the signal for each gene in infected DCs to the signal for uninfected iDCs. Table I shows particular genes for which expression was up-regulated, down-regulated, or unaffected by mycobacterial stimulation. Stimulation with *M. leprae* did not induce expression of any of the genes involved in DC maturation/activation and, in fact, some genes that were up-regulated by exposure to Mtb and/or BCG were down-regulated by *M. leprae*. Genes encoding MHC II and costimulator molecules such as CD80 and CD83, as well as IL-12 and TNF-α, were induced in DCs exposed to BCG or Mtb but not to *M. leprae*, confirming the observations made on cell-associated protein (surface marker) and on secreted protein (cytokines). Among the genes down-regulated by *M. leprae* but up-regulated by Mtb or BCG were IFN-γ inducible proteins and IL-2. IL-1β and NFκB were up-regulated in response to Mtb or BCG, consistent with numerous reports, and down-regulated by *M. leprae*. CCR7 was the only chemokine receptor expressed by DC. Mtb or BCG induced increased CCR7 expression, whereas *M. leprae* inhibited it. IFN-α was down-regulated by *M. leprae* but unaffected by Mtb or BCG. Expression of TLR9, which has recently been implicated as playing a role in the host response to mycobacteria (13), was up-regulated by Mtb or BCG but unaffected by *M. leprae*.

**Discussion**

We have found that *M. leprae* has a neutral or somewhat inhibitory effect on DC activation and maturation in vitro. This is in contrast to Mtb or BCG, both of which induce up-regulation of phenotypic maturation markers on DCs and enhance proinflammatory cytokine production. Interestingly, at the level of mRNA expression, *M. leprae* did not activate pathways that BCG or Mtb induced and, in fact, seemed to dampen the expression of many of the genes up-regulated by BCG or Mtb. We found that the lack of *M. leprae*-mediated DC maturation/activation resulted in reduced T cell priming as evaluated by a MLR. Despite this lack of maturation/activation and reduced T cell priming, there was a clear induction of Th2 type cytokines in the MLR assay when *M. leprae*-stimulated DCs were used as targets. However, Th1 cytokines were also induced. These observations may reflect an early coexistence of Th1 and Th2 responses that later become polarized in one or another direction, determining either T-lep or L-lep clinical/immunologic profiles. This mixed Th1/Th2 response might reflect incomplete DC activation, alternative activation (in the direction of DC2, which promotes Th2 polarizing signals), or suppression of DC activation. Indeed, iDCs and incompletely activated DCs have been associated with anergy and tolerance in other systems (14). Unmethylated CpG oligodeoxynucleotide motifs induce APC activation through TLR9 (15). They also induce DC production of indoleamine 2,3-dioxygenase (IDO), a strong immunosuppressive molecule that induces anergy (16). Indoleamine 2,3-dioxygenase has been implicated in immune tolerance in cancer and during pregnancy (17). *M. leprae* might behave like CpG, inducing both DC activation and immunosuppressiveness.

In contrast to our present study, other investigators have reported *M. leprae*-stimulated DC activation and maturation, but only at a very high MOI value of >40 (18). This dose-dependent effect might be explained by the possibility that DC-stimulating *M. leprae* molecules such as major membrane protein II are present in subcellular locations at concentrations that are relatively low (19, 20). It is likely that the effect of very low doses of stimulating factors is offset by inhibitory molecules exposed on the surface of the bacilli, ultimately resulting in a neutral cellular response. Defective IFN-γ mediated activation has been reported in mouse macrophages heavily infected with live *M. leprae* (21, 22). Possible inhibitory molecules present in the cell wall of *M. leprae* include phenolic glycolipid I (PGL-I) (23, 24). PGL-1 has been implicated in dampening the allogeneic response caused by *M. leprae*-infected DCs (18). It has also been implicated in reducing cytokine production in macrophages (25). Our analysis of DC-specific genes expressed in

Table I. BCG and Mtb induce and *M. leprae* suppresses expression of genes involved in DC activation and maturation

<table>
<thead>
<tr>
<th>Gene Family</th>
<th><em>M. leprae</em></th>
<th>Mtb</th>
<th>BCG</th>
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<tr>
<td>CCL3</td>
<td>0.78</td>
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<tr>
<td>CCL22</td>
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<td>2.56</td>
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<td>CCR7</td>
<td>0.49</td>
<td>2.01</td>
<td>2.13</td>
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<tr>
<td>CD80 (surface costimulator)</td>
<td>0.89</td>
<td>2.97</td>
<td>3.45</td>
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<tr>
<td>ICAM1</td>
<td>1.02</td>
<td>1.21</td>
<td>2.10</td>
</tr>
<tr>
<td>ICAM2</td>
<td>1.40</td>
<td>0.79</td>
<td>2.15</td>
</tr>
<tr>
<td>IFN-γ-inducible protein (IFI44)</td>
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<td>2.56</td>
<td>6.29</td>
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<td>MHC II</td>
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<tr>
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<td>1.51</td>
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<td>6.22</td>
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<td>0.33</td>
<td>7.31</td>
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<td>6.12</td>
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<td>TNF-α</td>
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<tr>
<td>TLR-9</td>
<td>0.82</td>
<td>10.30</td>
<td>9.69</td>
</tr>
</tbody>
</table>

A Fold difference between infection and iDC (all in the presence of IL-4 and GM-CSF) following 5 h of stimulation. Boldface, Differences considered significant; boldface and italics, significantly lower fold difference relative to iDC; boldface only, significantly higher fold difference relative to iDC. Results shown are representative of three experiments.
response to mycobacterial stimulation suggests there might be active suppression of maturation/activation pathways, which would be consistent with PGL-1 dampening activation signals. Alternatively, PGE₂, which has been implicated in suppressing IFN-γ-mediated activation of *M. leprae*-infected macrophages (26), could be polarizing DC into DC2 (27). The dampening effect of PGL-1 or PGE₂ might eventually be overcome at higher MOI values; the interplay between the activating and suppressing signals could determine the tipping point between the development of Th1- or Th2-type immunity.

Because of the difficulties of working with *M. leprae* i.e., the inability to grow the mycobacterium in vitro and the lack of an optimal animal model for pathogenesis studies, limited progress has been made in understanding the host-pathogen interactions that lead to the dramatic anergy of *M. leprae*-specific, cell-mediated immunity in lepromatous patients. Host-pathogen interactions for other mycobacteria, however, are better understood. By comparing cellular responses to well-characterized strains such as *M. tuberculosis* H37Rv and *M. bovis* BCG with responses to *M. leprae*, possible mechanisms that lead to mycobacterial modulation of cellular function can be explored. Various BCG and Mtb molecules that modulate APC activation have been described. One such mycobacterial component that has been extensively studied is a major cell wall component, lipomannobiose (LAM) (28). A recent report showed that lipomannobiose, the biosynthetic LAM precursor, displays strong proinflammatory effects via TLR-2 (29). Mannose-capped LAM (man-LAM) from slow growing pathogenic mycobacteria, in contrast, has been found to inhibit phagosome maturation, apoptosis, and IFN-γ signaling (30). *M. leprae* LAM has been reported to be a potent inhibitor of IFN-γ-mediated activation of mouse macrophages (26). Mannooligosaccharide caps on LAM have been shown to interact with the mannose receptor and to induce expression of IRAK-M, which interferes with TLR-mediated macrophage activation (31). Man-LAM has also been shown to bind the DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), a C-type lectin on DC and macrophages (32), and to interfere with TLR-mediated DC maturation (33). Interestingly, increased DC-SIGN expression in *M. leprae*-infected DCs has been recently described in L-lep vs T-lep patients (34). This report and a second one (35) showed that DC-SIGN acts as an entry receptor for *M. leprae*, much the same as has been described for *M. tuberculosis* (32, 36). Taken together, these data suggest that *M. leprae* might induce expression of its own receptor, which would provide access to a safe haven and a means to reduce Man-LAM/TLR mediated DC activation. These observations also suggest that the cell wall contains molecules that enhance and others that suppress APC activation and that the ratio of lipomannobiose to Man-LAM and other lipids such as PGL-1 and the availability of their receptors on DCs may determine the nature of the cellular response to exposure to the microorganism.

In summary, by using different assays that target different manifestations of DC maturation, this report provides evidence that *M. leprae* actively suppresses APC activation/maturation. This effect is probably an important determinant of T cell-dependent, Ag-specific responsiveness. Improved understanding of the mechanism by which *M. leprae* both inhibits and enhances early immunologic events that affect acquired immunity should contribute to improved understanding of how immune anergy vs immune activation are regulated in leprosy as well as other human diseases.

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References


