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Mycobacterium tuberculosis Induces Differential Cytokine Production from Dendritic Cells and Macrophages with Divergent Effects on Naive T Cell Polarization

Sonia Perdow Hickman, John Chan, and Padmini Salgame

Th1-mediated cellular responses are important for protection in tuberculosis. However, the mechanisms and APC types responsible for initiating Th1 responses are not well understood. These studies show that macrophages and dendritic cells, albeit both being APC, respond differently following Mycobacterium tuberculosis infection and thereby have different consequences for the development of naive T cells. We report that M. tuberculosis-infected dendritic cells bias the polarization of OVA peptide-specific naive transgenic T cells to the Th1 phenotype, and, in contrast, in the presence of infected macrophages naive T cells do not develop a Th1 phenotype. Comparison of the cytokine profile expressed by the infected dendritic cells and macrophages revealed several differences, the most striking being that infected macrophages did not express the Th1-promoting cytokine IL-12. These studies also show that IL-10 is responsible for the failure of IL-12 production by M. tuberculosis-infected macrophages, and that the effects of IL-10 can be overcome by IFN-γ priming. We speculate that the observed difference in response of the two APC types to M. tuberculosis infection may be a reflection of their respective roles in immune initiation and granuloma regulation.

W
ith one-third of the world’s population infected with Mycobacterium tuberculosis, tuberculosis (TB) remains a major threat to public health (1). Existing evidence suggests that cellular Th1-mediated immunity plays a critical role in host defense against M. tuberculosis. Mice with disruption of the IFN-γ gene exhibit deficient production of the antimycobacterial reactive nitrogen intermediates and have defective granuloma formation; consequently, they are highly susceptible to M. tuberculosis infection (2, 3). IL-12, a cytokine critical for the development of Th1 cells, is essential for protection against M. tuberculosis in murine experimental TB models (4, 5). In humans, strong evidence for the importance of IFN-γ (6, 7) and IL-12 (8, 9) in host defense against mycobacterial infection has recently emerged. The protective role of Th1 cell-mediated immune response in TB is perhaps best illustrated by the enhanced susceptibility to M. tuberculosis in individuals with HIV infection, a disease characterized by profound loss of CD4+ T cells, including the Th1 subtype (10). Therefore, while it is well established that the protective immune response to M. tuberculosis is dependent on the host’s ability to initiate Th1 cellular responses, the microenvironment essential for the development of these Th1 cells is not well understood.

Among several factors that can regulate naive T cell polarization and development of Th1 or Th2 type in response to infection, the APC is perhaps the most influential. Since its initial identification as a novel cell type in peripheral lymphoid organs of mice (11), dendritic cells have gained acceptance as potent APC. While macrophages and dendritic cells can both process and present Ag to T cells, dendritic cells are unique in their ability to initiate a primary immune response. This functional feature of the dendritic cell is due to its high mobility and high surface expression of MHC and costimulatory molecules. In response to antigenic stimuli, immature dendritic cells migrate rapidly to peripheral tissue, where they can detect, capture, and process foreign Ag. Following Ag encounter, dendritic cells fully mature into APC, up-regulate their chemokine receptors, and migrate into T-cell-enriched areas of lymphoid tissue. Here the Ag-loaded dendritic cells present Ag-derived peptides associated with either class I or class II MHC molecules to naive CD8+ and CD4+ T cells, respectively (12–14). Dendritic cell maturation is also regulated by proinflammatory cytokines of the innate immune response such as TNF-α and IL-1β (13, 15, 16).

M. tuberculosis resides mainly within macrophages; however, recent studies have shown that dendritic cells can also phagocytose the bacterium (17, 18). In addition, both these APC types are present in the lung (18) and are activated following infection (19). Thus, upon infection both cell types can potentially regulate the polarization of naive T cells. Therefore, in this study we examined whether following M. tuberculosis infection dendritic cells and macrophages differ in their ability to influence the polarization of naive T cells obtained from spleens of OVA peptide TCR-transgenic mice. We demonstrate that dendritic cells infected with M. tuberculosis direct the development of naive Th cells toward the Th1 phenotype due to their potential to produce IL-12 in the presence of microbial stimuli alone. In contrast, infected macrophages do not secrete IL-12 and consequently are not capable of supporting naive T cell development toward a Th1 phenotype.

Materials and Methods

Mice

Female BALB/c were purchased from The Jackson Laboratory (Bar Harbor, ME). OVA peptide-specific DO11.10 TCR-transgenic mice on the

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3Abbreviations used in this paper: TB, tuberculosis; MOI, multiplicity of infection; BMMD, bone marrow-derived macrophage; BMDC, bone marrow-derived dendritic cell.

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BALB/c background were generated by Dr. K. Murphy (Washington University School of Medicine, St. Louis, MO) (20) and obtained from Dr. R. Seder (National Institutes of Health, Bethesda, MD). For these studies transgenic mice were bred and maintained under pathogen-free conditions at the Rodent Barrier Facility of Temple University School of Medicine (Philadelphia, PA). IL-10-deficient mice on the BALB/c background were a gift from Dr. C. A. Hunter (University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA).

Microbial stimuli

The virulent M. tuberculosis Erdman strain (Trudeau Institute, Saranac Lake, NY), obtained after mouse passage, was grown in culture, titrated, and stored in aliquots at 70°C. Before infection, aliquots were thawed, briefly sonicated, and then added to cultures at the appropriate multiplicity of infection (MOI). Escherichia coli LPS was purchased from Sigma-Aldrich (St. Louis, MO).

Macrophages and dendritic cells

Bone marrow was flushed from the femora and tibiae of mice and cultured in DMEM/10% FCS supplemented with 20% L cell-conditioned medium as previously described (21). Bone marrow-derived macrophages (BMMøs) were harvested on day 7. For obtaining bone marrow-derived dendritic cells (BMDCs), bone marrow cells were cultured at a density of 10^6 cells/ml in DMEM/10% FCS supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM), 2-ME (5 x 10^-5 M), and 750 μM murine eG-CSF (R&D Systems, Minneapolis, MN). Every 2 days one-half of the medium was removed and supplemented with fresh medium and eG-CSF. Nonadherent cells comprising the immature dendritic cell population were harvested on day 7. In some experiments adherent splenic macrophages were obtained from spleen cell suspensions by adherence for 1 h to tissue culture-treated plates. Adherent cells were harvested by incubation with 5 mM EDTA in Ca^2+ - and Mg^2+-free PBS for 10 min. The purity of each population was determined by FACS analysis of specific surface markers and ranged from 80 to 95%.

Direct isolation of splenic dendritic cells and macrophages

Spleen cell suspensions were prepared by collagenase digestion with collagenase VII (Sigma-Aldrich) that was endotoxin free. Splenic dendritic cells were enriched by MACS with anti-CD11c magnetic beads (Miltenyi Biotec, Auburn, CA) using positive selection columns. CD11c^-cells were used to isolate CD11b^+ splenic macrophages by MACS with anti-CD11b magnetic beads. The enriched populations were subjected to FACS analysis for surface expression of CD11b and CD11c, and purity ranged from 80 to 95%.

Isolation of transgenic T cells

Naive transgenic T cells were isolated from the spleens of DO11.10 transgenic mice. T cells were purified by removing macrophages and B cells from spleen cell cultures by adherence to plastic, followed by adherence to nylon wool columns (Polysciences, Warrington, PA) (22). We routinely observed that >90% of the isolated T cells were CD4^+.

Cytokine assays

Analysis of cytokines present in supernatants was performed by ELISA using Ab pairs from BD Pharmingen (San Diego, CA): C15.6 and C17.8 (biotinylated) for IL-12p40, 9A5 and C17.8 (biotinylated) for IL-12p70, JES5-2A5 and JES5-16E3 (biotinylated) for IL-10, R4-62A and XMG1.2 (biotinylated) for IFN-γ, 1B11 and BV6-24G2 (biotinylated) for IL-4, and G281-2626 and MP6-XTB for TNF-α.

RNase protection assays

Total RNA was isolated from cells using RNAzol B reagent (Biotecx Labortoratories, Houston, TX) according to the manufacturer’s protocol. The RNase protection assay was performed using 7–10 μg total RNA and the Riboquant kit (BD Pharmingen). Protected [^32P]UTP-labeled probes were resolved on a 5% polyacrylamide gel, analyzed by autoradiography, and quantitated by phosphorimaging using MultiAnalyset Systems software (Bio-Rad, Hercules, CA).

Flow cytometry for IL-10R expression

IL-10R expression was measured by immunofluorescence. Cells (1 x 10^6) were infected with M. tuberculosis (3 MOI) in the presence of neutralizing Ab of the same isotype. Surface fluorescence was assayed by flow cytometry.

Statistics

For statistical analysis of samples, paired and unpaired Student’s t tests were performed (PRISM version 3.0; GraphPad, San Diego, CA); a value of p < 0.05 was considered significant.

Results

M. tuberculosis-infected dendritic cells polarize naive T cells to Th1 type

We delineated the capacity of M. tuberculosis-infected macrophages and dendritic cells to bias Ag-specific naive T cells to effector Th1 and Th2 cells. A difficulty in studying naive T cell differentiation is the low number of Ag-specific naive T cells present during immune initiation. Therefore, we took advantage of the DO11.10 αβ TCR-transgenic mice that express a TCR-αβ specific for OVA peptide, aa 323–339 presented on MHC molecule I-A^d. T cells from these mice were used to study the effect on priming by M. tuberculosis-infected dendritic cells and macrophages.

Dendritic cells and macrophages were infected at different MOIs for 24 h. Naive T cells obtained from the spleens of transgenic mice were allowed to differentiate in the presence of OVA peptide in an M. tuberculosis-infected APC milieu for 72 h. T cells were then re-exposed to Ag and APC in the absence of any biasing modalities, and the cytokine profile of the differentiated T cells was determined. As observed in Fig. 1, in the absence of any M. tuberculosis infection neither dendritic cells nor macrophages had any polarizing effect on the differentiating naive T cells. However, when naive T cells were allowed to differentiate in the presence of M. tuberculosis-infected dendritic cells they acquired Th1 polarity. In contrast, naive T cells differentiating in the M. tuberculosis-infected macrophage setting failed to differentiate into Th1 cells and continued to maintain a Th0 phenotype. This differential response on T cell polarization by dendritic cells and macrophages was seen at all three MOIs tested (Fig. 1A).

In the above experimental setup, M. tuberculosis was allowed to interact with APCs for 24 h before addition of T cells and OVA peptide, thereby biasing the system toward soluble factors being prime inducers of T cell polarity. To further confirm the soluble nature of the polarizing factor we used a Transwell system. In this experimental system the infected cells are separated from irradiated splenic APC, OVA peptide, and transgenic T cells by a 0.4-μm membrane. Any changes in T cell polarity can be assumed to be mediated by the release of soluble mediators from the M. tuberculosis-infected APCs. As observed previously in the absence of infection, dendritic cells had no effect on T cell polarity. However, following M. tuberculosis infection they were able to induce a Th1 polarity to the differentiating naive T cells. These data suggest that soluble factors released from M. tuberculosis-infected dendritic cells were responsible for imposing the Th1 polarity on naive T cells. In contrast, soluble factors released from M. tuberculosis-infected macrophages did not influence naive T cell differentiation, and, as observed previously, the differentiated T cells maintained their Th0 polarity (Fig. 1B).

IL-12 released from dendritic cells is critical for naive T cell polarization to Th1 type

Because previous studies have determined that IL-12 is a critical factor for Th1 cell responses (23), we consequently examined the secreted levels of IL-12p40 from M. tuberculosis-infected dendritic cells and macrophages. M. tuberculosis infection of dendritic cells induced significant levels of IL-12p40 production at all three MOIs tested. However, the same infection level in macrophages...
induced very low levels of IL-12p40 (Fig. 2A). Interestingly, parallel evaluation for TNF-α levels revealed that *M. tuberculosis* infection induced similar levels of this cytokine in both dendritic cells and macrophages (Fig. 2B). These data indicated that the interaction of *M. tuberculosis* and macrophage resulted in production of the proinflammatory cytokine TNF-α, ruling out that lack of IL-12p40 production was due to lack of signaling from macrophages. We next examined the level of bioactive IL-12p70 and noted a similar differential response; dendritic cells produced IL-12p70 following *M. tuberculosis* infection, whereas infected macrophages did not secrete any detectable levels of the cytokine (Fig. 2C). The difference in IL-12p40 protein expression was also seen at the level of gene transcription (Fig. 2D). There was a 74% induction of mRNA levels for IL-12p40 compared with housekeeping gene L32 in dendritic cells following infection. In contrast, only a 2.2% increase was observed in macrophages.

The studies described above were performed with dendritic cells and macrophages derived in vitro from bone marrow cells cultured under the influence of different combinations of growth factors. To eliminate in vitro culturing effects on cytokine production, we also tested IL-12p40 production from dendritic cells and macrophages that were directly isolated from spleens. *M. tuberculosis* infection of directly isolated CD11c<sup>+</sup> dendritic cells and CD11b<sup>+</sup> macrophages from spleens exhibited the same phenotype for IL-12p40 production as seen with the bone marrow-derived cells (Fig. 3A). Also as previously observed, IL-4 production was suppressed in T cells biased under the influence of *M. tuberculosis*-infected CD11c<sup>+</sup> dendritic cells; however, similar suppression of IL-4 production was not observed in T cells biased under the influence of CD11b<sup>+</sup> macrophages (Fig. 3B). Thus, the biasing properties of BMDCs and macrophages were replicated in the directly isolated dendritic cells and macrophages. CD11c<sup>+</sup> dendritic cells supported Th1 responses, whereas CD11b<sup>+</sup> macrophages were unable to do so.

To further evaluate the critical role of IL-12 produced by *M. tuberculosis*-infected dendritic cell in T cell differentiation, we examined the ability of dendritic cells to polarize naive T cells in the presence of neutralizing anti-IL-12p40 Abs. Indeed, in the presence of neutralizing Abs, dendritic cells secreted significantly lower amounts of IFN-γ (Fig. 2E), confirming that IL-12 produced by *M. tuberculosis*-infected dendritic cells was actually responsible for polarizing naive T cells to Th1. Together, the data presented in Figs. 2 and 3 support that microbial stimulation is sufficient to induce IL-12 production from dendritic cells, which can then influence naive T cell polarization to the Th1 type.

### IL-12 PRODUCTION BY *M. tuberculosis*-INFECTED DENDRITIC CELLS

![Diagram](http://www.jimmunol.org/)

**FIGURE 1.** *M. tuberculosis*-infected dendritic cells polarize naïve T cells to Th1-type. A, BMDCs (2 × 10<sup>5</sup>) and adherent splenic macrophages (both cell types were derived from BALB/c mice) were either uninfected (None) or infected with the virulent Erdman strain of *M. tuberculosis* at MOIs of 10, 3, and 1. Naive D011.10 TCR-transgenic T cells (0.5 × 10<sup>5</sup>) and OVA peptide (0.3 μM) were added to the cultures 24 h later. On day 7 T cells were harvested and stimulated (2 × 10<sup>5</sup>) with peptide-pulsed irradiated splenocytes (5 × 10<sup>5</sup>), but in the absence of *M. tuberculosis*. Forty-eight hours later supernatants were harvested and analyzed for IFN-γ and IL-4 by sandwich ELISA. The experiments described used splenic macrophages as the macrophage population because BMDC did not present the OVA peptide efficiently to T cells in the absence of IFN-γ priming. Data are representative of one of two individual experiments (***, p < 0.0001). B, BMDCs (1 × 10<sup>5</sup>), splenic macrophages, or BMDC were either untreated or infected with *M. tuberculosis*. Twenty-four hours later the Transwell was placed into the chamber. Naive T cells (0.5 × 10<sup>5</sup>), OVA peptide (0.3 μM), and irradiated adherent splenocytes (0.3 × 10<sup>5</sup>) were added into the Transwell. IL-2 was then added on day 3 to the cultures. On day 7 poststimulation, T cells were harvested and restimulated with peptide in the absence of *M. tuberculosis* (nonbiasing condition). Supernatants were harvested 48 h later and analyzed for IFN-γ and IL-4 by sandwich ELISA. Note that in this experimental scheme the test APCs are not presenting Ag, but the effects of factors secreted from them following infection induced similar levels of this cytokine in both dendritic cells and macrophages. *M. tuberculosis* infection of directly isolated CD11c<sup>+</sup> dendritic cells and CD11b<sup>+</sup> macrophages from spleens exhibited the same phenotype for IL-12p40 production as seen with the bone marrow-derived cells (Fig. 3A). Also as previously observed, IL-4 production was suppressed in T cells biased under the influence of *M. tuberculosis*-infected CD11c<sup>+</sup> dendritic cells; however, similar suppression of IL-4 production was not observed in T cells biased under the influence of CD11b<sup>+</sup> macrophages (Fig. 3B). Thus, the biasing properties of BMDCs and macrophages were replicated in the directly isolated dendritic cells and macrophages. CD11c<sup>+</sup> dendritic cells supported Th1 responses, whereas CD11b<sup>+</sup> macrophages were unable to do so.

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**M. tuberculosis selectively suppresses IL-12 production by macrophages**

Having shown that *M. tuberculosis* induces strikingly different responses from dendritic cells and macrophages with regard to IL-12 production, we next determined whether *M. tuberculosis* was specifically inhibiting the production of IL-12 by macrophages. The data presented in Fig. 4C show that if macrophages are stimulated with IFN-γ and LPS they produce 1.2 ng/ml IL-12p40. However, if the macrophages are pre-exposed to *M. tuberculosis* for 6 h before the addition of IFN-γ and LPS, IL-12p40 production is significantly suppressed (*p < 0.005). The selectivity of the *M. tuberculosis*-induced suppressive effect was tested in parallel against TNF-α. Supernatants of stimulated macrophages with or without prior exposure to *M. tuberculosis* had similar levels of TNF-α (Fig. 4D), suggesting that *M. tuberculosis* had a selective suppressive effect on IL-12p40 production. *M. tuberculosis*-infected dendritic cells, in contrast, did not exhibit a similar significant suppression of IL-12p40 production in response to IFN-γ and LPS stimuli (Fig. 4A).

**IL-10 is responsible for suppressing IL-12p40 production from *M. tuberculosis*-infected macrophages**

IL-10 is an immunoregulatory cytokine with potent immunosuppressive activity against both APC and Th1 cells (24). IL-10 also potently inhibits the production of several proinflammatory cytokines from macrophages, including IL-12 production (24, 25). IL-12 production by dendritic cells is also down-regulated by
IFN-γ priming synergizes with M. tuberculosis stimuli to elicit IL-12 production from macrophages

The T cell-derived signals IFN-γ (28) and CD40 ligand (29) both exert a potent synergistic effect on IL-12p40 induction in macrophages stimulated with LPS. Therefore, we tested whether addition of IFN-γ with M. tuberculosis would induce IL-12p40 induction from macrophages. Cells were treated with IFN-γ and M. tuberculosis, and 24 h later supernatants were harvested and analyzed by ELISA for IL-12p40. IL-12p40 protein was secreted by macrophages when stimulated with both M. tuberculosis and IFN-γ (Fig. 7). In addition, there was a conspicuous abrogation of IL-10 production when macrophages were infected with M. tuberculosis in the presence of IFN-γ (Fig. 7). In dendritic cells IFN-γ treatment further up-regulated IL-12p40 levels (Fig. 7). These data indicate that in addition to microbial stimuli, macrophages require a second T cell-derived signal to elicit IL-12p40 induction. The mechanism underlying the requirement for a T cell-derived signal appears to be at the level of IL-10 inhibition.

Discussion

The polarization of naive T cells to effector Th1 and Th2 subtypes is greatly influenced by the APC and the cytokines they make following microbial stimulation. M. tuberculosis can infect both macrophages and dendritic cells equally efficiently (17, 18). To better understand immune initiation to M. tuberculosis, we used an in vitro cell model system to study pathogen-induced dendritic cell and macrophage activation and subsequent effects on Th1 development from naive T cells. Our results show that M. tuberculosis-infected dendritic cells
induce the development of naive Th0 cells into Th1 effector cells. In contrast, infected macrophages had no influence on the polarity of maturing naive T cells. The differential capacity to affect naive T cell development by the two APC types is due to unique interactions of \( M.\) \( \text{tuberculosis} \) with dendritic cells.

Immature dendritic cells can be modulated to become mature dendritic cells with contrasting phenotypes that can polarize naive T cells in different directions. A subset of mature dendritic cells, for instance, secretes high levels of IL-12 and selectively enhances polarization toward the Th1 phenotype (30). In contrast, an IL-10-secreting dendritic cell subset induces Th2 responses and T cell tolerance (31). Therefore, it was expected that \( M.\) \( \text{tuberculosis} \)-infected dendritic cells that were potent inducers of Th1 responses

**FIGURE 3.** Dendritic cells directly isolated from spleens are also efficient at inducing Th1 responses. A, CD11c\(^+\) DC or CD11b\(^+\) macrophages (M\( \phi \); 0.25 \( \times \) 10\(^6\) cells/ml) were left untreated or were infected with \( M.\) \( \text{tuberculosis} \). Supernatants were harvested 48 h later and analyzed for IL-12p40. B, CD11c\(^+\) DC or CD11b\(^+\) macrophages (M\( \phi \); 2 \( \times \) 10\(^7\)) were infected with \( M.\) \( \text{tuberculosis} \) for 8 h and then pulsed with OVA peptide. Naive T cells (2 \( \times \) 10\(^5\)) were then added and cultured for 7 days. Following primary stimulation, T cells were restimulated under nonbiasing conditions as previously described. T cell phenotypes were analyzed by ELISA. Data are expressed as the percent suppression of cytokine production and are calculated as follows: 100 - (levels of cytokine in experimental culture/levels of cytokine in control peptide alone cultures) \( \times \) 100. Results are representative of one of two individual experiments.

**FIGURE 4.** \( M.\) \( \text{tuberculosis} \) suppresses IL-12p40 production by macrophages. BMDCs (\( A \) and \( B \)) or BMM\( \phi \) (\( C \) and \( D \)) were left untreated or were infected with \( M.\) \( \text{tuberculosis} \) for 6 h. Culture medium was removed, and fresh medium containing IFN-\( \gamma \) (100 U/ml) was added where indicated. Cells were primed with IFN-\( \gamma \) for 5 h, following which LPS (100 ng/ml) was added to the cultures. Supernatants were harvested 16 h after the addition of LPS and assayed for IL-12p40 (\( A \) and \( C \)) and TNF-\( \alpha \) (\( B \) and \( D \)) production. Data shown are the means \( \pm \) SD of triplicate samples (*, \( p < 0.05; **, p < 0.005 \)).

**FIGURE 5.** \( M.\) \( \text{tuberculosis} \) induces IL-10 in both dendritic cells and macrophages following infection. A, IL-10 levels were measured in the supernatants of untreated (UT) BMDCs and BMM\( \phi \) (0.5 \( \times \) 10\(^6\) cells/ml) and cells infected with \( M.\) \( \text{tuberculosis} \) for 24 h. Results are the means \( \pm \) SD of triplicate samples and are representative of four independent experiments. B, BMDCs and BMM\( \phi \) were infected with \( M.\) \( \text{tuberculosis} \) (3 MOI). After 12 h cells were harvested and stained with Abs to the IL-10R or with an irrelevant Ab. Surface expression of IL-10R was measured by flow cytometry. Data are representative of two individual experiments.
would secrete high levels of IL-12 and no IL-10, as seen previously with other diverse stimulating conditions (32–36). Surprisingly, M. tuberculosis-infected dendritic cells secreted both IL-12 and IL-10 but were nevertheless able to initiate Th1 responses from naive T cells. Our data contrast with those demonstrating that IL-10-secreting dendritic cells, in fact, induce immune tolerance (31). We believe that the reason that M. tuberculosis-infected dendritic cells possess Th1-promoting ability may be due to a rapid induction of IL-12 following infection. Once IL-12 is produced, the presence of IL-10 does not have a down-modulating effect on Th1 polarization. This has been reported previously, where exogenous addition of IL-12 biased naive T cells to the Th1 type, and exogenous addition of both IL-12 and IL-10 still resulted in naive T cell differentiation to the Th1 type (37). Future studies will address the kinetics of IL-12 and IL-10 production by dendritic cells and macrophages following M. tuberculosis infection. As previously suggested (38), these data also indicate that dendritic cells do not have an intrinsic attribute for Th1 priming, but have to be environmentally instructed and influenced to acquire Th1 priming capacity.

Contrary to what we observed with dendritic cells, macrophages only secreted IL-10 with no detectable levels of IL-12 following M. tuberculosis infection. Because previous studies have shown that IFN-γ can synergize with LPS to induce IL-12p40 production from macrophages, we examined whether IFN-γ would similarly synergize with M. tuberculosis to induce IL-12p40 from macrophages. Indeed, stimulation of macrophages with both IFN-γ and M. tuberculosis induced IL-12p40 production, and notably this was associated with a reciprocal down-regulation of IL-10 production. That IL-10 is indeed responsible for inhibiting IL-12 production from macrophages was proved when we observed that macrophages from IL-10 knockout mice were enabled to secrete IL-12 and support Th1 responses. Interestingly, in the absence of endogenous IL-10, a further enhancement of IL-12 production occurred in dendritic cells following infection, suggesting that IL-10 production by dendritic cells subsequent to IL-12 production may be important for tempering dendritic cell responses and preventing excessive proinflammatory responses and ensuing tissue damage.

We speculate that the capacity of dendritic cells, but not macrophages, to synthesize IL-12 in response to only microbial triggering may have functional significance for the two APC types during M. tuberculosis infection. Dendritic cells are present in the respiratory tract (39) and have been shown to quickly transport Ag from the airways to the lymph nodes for T cell priming (40). During this phase of innate immune response it is important that dendritic cells rapidly make IL-12 in response to triggering by M. tuberculosis alone. In contrast, macrophages may have a more important role in the tuberculous granuloma, where they are in close apposition with activated T cells. In this microenvironment IFN-γ secreted by activated T cells can synergize with M. tuberculosis to induce IL-12p40 from macrophages. Indeed, stimulation of macrophages with both IFN-γ and M. tuberculosis induced IL-12p40 production, and notably this was associated with a reciprocal down-regulation of IL-10 production. That IL-10 is indeed responsible for inhibiting IL-12 production from macrophages was proved when we observed that macrophages from IL-10 knockout mice were enabled to secrete IL-12 and support Th1 responses. Interestingly, in the absence of endogenous IL-10, a further enhancement of IL-12 production occurred in dendritic cells following infection, suggesting that IL-10 production by dendritic cells subsequent to IL-12 production may be important for tempering dendritic cell responses and preventing excessive proinflammatory responses and ensuing tissue damage.

FIGURE 6. Macrophages from IL-10 knockout, but not wild-type, mice infected with M. tuberculosis can promote Th1 cell polarization. A. BMDCs and BMMΦ from wild-type (WT) or IL-10−/− mice were infected with M. tuberculosis. Twenty-four-hour supernatants were analyzed for the presence of IL-12p70. B, M. tuberculosis-infected BMDCs and BMMΦ derived from both wild-type and IL-10−/− mice were tested for their ability to bias naive T cells by the Transwell method described above. Data shown are the means ± SD of triplicate samples.

FIGURE 7. IFN-γ down-regulates IL-10 production from M. tuberculosis-infected macrophages. BMDCs and BMMΦ were stimulated with M. tuberculosis alone or with M. tuberculosis and IFN-γ (100 U/ml). Supernatants were harvested 24 h later and measured for IL-12p40 and IL-10. Results are representative of two independent experiments and are the means ± SD of triplicate samples (*, p < 0.05; **, p < 0.005).
First, only *M. tuberculosis*-infected DCs support Th1 priming because of their unique ability to make IL-12 in response to *M. tuberculosis* infection, and, second, mechanically the failure of *M. tuberculosis*-infected macrophages to synthesize IL-12 is due to IL-10. The fact that dendritic cells are able to synthesize sufficient bioactive IL-12 for Th1 priming despite the production of IL-10 underscores the complexity of the molecular mechanisms regulating IL-12 gene expression in the two APC types. The differences in response of the two types of APCs to *M. tuberculosis* could be a reflection of their respective roles in immune initiation to *M. tuberculosis* and granuloma regulation. We promote the concept that dendritic cell captures Ags of *M. tuberculosis* and transport it to the lymph nodes for T cell priming and Th1 polarization, because they are the primary secretors of IL-12 following *M. tuberculosis* infection. In contrast, macrophages are important for micobacterial function in the granuloma because they are more efficient in killing intracellular *M. tuberculosis* (17) and for sustaining Th1 polarity. In summary, the present study strongly positions the IL-12-secreting dendritic cell as the bridge between innate and adaptive immunities in TB, with important implications for dendritic cell-based vaccine design strategies (19, 42).

References