IL-10 Converts Human Dendritic Cells into Macrophage-Like Cells with Increased Antibacterial Activity Against Virulent Mycobacterium tuberculosis

Daniela Förtsch, Martin Röllinghoff and Steffen Stenger

*J Immunol* 2000; 165:978-987;
doi: 10.4049/jimmunol.165.2.978
http://www.jimmunol.org/content/165/2/978

References
This article cites 69 articles, 40 of which you can access for free at:
http://www.jimmunol.org/content/165/2/978.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-10 Converts Human Dendritic Cells into Macrophage-Like Cells with Increased Antibacterial Activity Against Virulent Mycobacterium tuberculosis

Daniela Förtsch, Martin Röllinghoff, and Steffen Stenger

Dendritic cells (DC) are unique in their ability to initiate a primary immune response by the presentation of soluble Ags to T cells. Recent studies have shown that DC also phagocytose particulate Ags including the intracellular pathogen Mycobacterium tuberculosis. However, it is not known whether DC contain the growth of intracellular organisms or allow unlimited replication. To address this question, we infected human DC with a virulent strain of M. tuberculosis and monitored the intracellular growth. The bacteria grew two orders of magnitude within 7 days of culture. Among cytokines known to modulate mycobacterial growth particularly in murine macrophages (TNF-α, IFN-γ, TGF-β, IL-4), only IL-10 modulated the growth in human DC. This effect was specific for immature dendritic cells, as IL-10 did not induce growth inhibition in human macrophages. In searching for the mechanism of growth inhibition, we found that IL-10 induces the down-regulation of the DC marker CD1, while the macrophage marker CD14 was up-regulated. Functionally, IL-10-treated cells had a reduced capacity to induce an alloresponse, but phagocytic uptake of M. tuberculosis was more efficient. We also show that DC are inferior to macrophages in containing mycobacterial growth. These findings show that IL-10 converts DC into macrophage-like cells, thereby inducing the growth inhibition of an intracellular pathogen. At the site of a local immune response, such as a tuberculous granuloma, IL-10 might therefore participate in the composition of the cellular microenvironment by affecting the maturity and function of DC. The Journal of Immunology, 2000, 165: 978–987.

M. tuberculosis, the causative agent of tuberculosis, is a facultative intracellular bacterium that has evolved sophisticated mechanisms to allow its survival inside host mononuclear phagocytes. Although M. tuberculosis was discovered by Koch over a century ago (1), the precise resistance mechanisms to this pathogen have not yet been resolved. An efficient cellular immune response is critical for preventing the development of clinically overt tuberculosis (2). One major mediator of protective immunity is IFN-γ, as mice or humans that lack components of the IFN-γ signaling pathway are highly susceptible to mycobacterial infection (3). Nevertheless, in vitro studies that involve human macrophages fail to demonstrate the activation of antimycobacterial effects by IFN-γ (4, 5). Thus, the activation of macrophages by IFN-γ is necessary, but not sufficient, for eradication of intracellular microorganisms. Therefore, additional mechanisms must be involved in the clearance of these organisms, particularly at the site of primary infection.

M. tuberculosis infects humans primarily via the respiratory route. Inhaled particles that are small enough to gain access to the terminal alveoli (<5 μm) are phagocytosed, processed, and presented mainly by alveolar macrophages (6). Another cell type present in the bronchoalveolar space, the airway epithelium, and lung parenchyma are dendritic cells (DC) (7, 8). Immature pulmonary DC are strategically located in pulmonary airways and distal alveoli, where they may function as sentinels for inhaled pathogens. Recent reports indicate an increased trafficking of DC into mucosal tissues in response to local bacterial stimuli such as Moraxella, Bordetella or Mycobacterium bovis bacillus Calmette-Guérin (BCG) (9–11). This property is likely to be central to the role of mucosal DC in surveillance of these front-line tissues for incoming microbial pathogens.

More recently, it has been shown that DC can internalize live pathogens including BCG (12), M. tuberculosis (13, 14), Bordetella bronchiseptica (15), Chlamydia trachomatis (16, 17), Borrelia burgdorferi (18), Trypanosoma cruzi (19), and Leishmania major (20). Although the in vivo interaction of intracellular microorganisms and lung DC is essential for priming naïve T cells, the ability of bacteria to survive in DC has not been elucidated. Human DC infected in vitro with M. tuberculosis or BCG undergo activation and maturation (13, 21, 22) that presumably enhance their potential to stimulate T cells. Also, murine DC infected with BCG and injected into the foot pad of mice can induce a specific T cell response and protect mice against mycobacterial aerosol challenge with M. tuberculosis (23). These findings support the hypothesis that DC strengthen the cellular immune response against mycobacteria.

We designed experiments to investigate whether DC also participate in protective immunity by mediating direct antimycobacterial activity. Growth of intracellular bacteria was investigated in the absence or presence of cytokines, which had previously been shown to modulate mycobacterial growth in murine macrophages. Among a variety of cytokines tested, only IL-10, a product of

1 Address of correspondence and reprint requests to Dr. Steffen Stenger, Friedrich-Alexander Universität Erlangen-Nürnberg, Institut für Klinische Mikrobiologie, Immunologie und Hygiene, Wasserturmstrasse 3, D-91054 Erlangen, Germany. E-mail address: stefen.stenger@mikrobio.med.uni-erlangen.de

2 Abbreviations used in this paper: DC, dendritic cell(s); MOI, multiplicity of infection; BCG, bacillus Calmette-Guérin; rhu, recombinant human.
macrophages with normally immunosuppressive effects, decreased the intracellular growth of \textit{M. tuberculosis}. In parallel, IL-10-treat-
m ent reduced the ability of DC to present mycobacterial lipid Ags to CD1-restricted T cells. This differential activity of IL-10 on DC may provide a mechanism to maintain the balance between a pro-
tective immune response and excessive cellular activation in the mi-
acroenvironment of a tuberculous granuloma.

Materials and Methods

Cell culture reagents

Cells were cultured in RPMI 1640 (Biochrom KG, Berlin, Germany) sup-
plemented with 10% heat-inactivated FCS (Sigma, St. Louis, MO), glu-
tamine (2 mM, Sigma), 10 nM HEPES, 13 mM NaHCO$_3$, 100 µg/ml streptomycin, and 60 µg/ml penicillin (all purchased from Biochrom). Ex-
periments involving the infection of cells with \textit{M. tuberculosis} were per-
formed in the absence of antibiotics, and FCS was replaced by pooled
human serum (generated from the blood of healthy volunteers) to optimize
formed in the absence of antibiotics, and FCS was replaced by pooled

Infection of DC

DC were infected with single-cell suspensions of \textit{M. tuberculosis}. After 4 h of
infection at 37°C, DC were harvested (slightly adherent cells were det-
bachy by trypan pipetting) and centrifuged at 800 rpm for 8 min. This
low-speed centrifugation selectively spins down DC while extracellular
bacteria remain in the supernatant. After three cycles of centrifugation,
the majority of extracellular bacteria were removed as determined by au-
rame- Rhodamine stain (TB-fluo, Merck, Darmstadt, Germany). Infected
cells were then plated at a concentration of 1 x 10$^6$ cells/ml in a 24-well plate
in culture medium without antibiotics plus 10% human serum. The effi-
ciency of infection, as quantitated by staining of control cultures on
Pernoxan chamber slides (Nunc) in every experiment was dependent on
the MOI. The microscopic evaluation of infected macrophages under the
fluorescence microscope confirmed the absence of any mycobacterial ag-
gregates. Cell viability of infected DC was determined by trypan blue
exclusion and was >99% in all experiments.

Cytokine treatment of cell cultures

Dendritic cells or macrophages were harvested, washed, and plated into
issue cultures plates. After the pulse infection with mycobacteria, cyto-
kines or anti IL-10 were added. The cytokines were not renewed (with
the exception of one experimental series) and were present throughout
the incubation period.

Confocal laser microscopy

Mycobacteria were incubated with an Ab directed against lipopatabinom-
mann (1:1 dilution of pure hydridoma supernatant) diluted in PBS/1% BSA/
20%FCS for 30 min at room temperature. Lipopatabinomann was labeled
by incubation of a 1:50 dilution (PBS/1% BSA/20%FCS) of a Texas Red-
d conjugated goat-anti-mouse Ab for 30 min. After an additional wash-
step, stained mycobacteria were resuspended and used for infection of DC,
which were cultured on Pernoxan chamber slides. After 4 h, nonphago-
cytosed bacteria were removed with PBS, and infected cells were stained with a FITC-conjugated Ab recognizing CD1a (1:20 dilution,
30 min, room temperature). Finally, cells were fixed with 4% paraformal-
dehyde (Sigma), mounted (Aquatax, Merck), and analyzed using a confo-
cal microscope (Leica, Solms, Germany).

Quantification of mycobacterial growth

To ensure the reliable quantification of intracellular \textit{M. tuberculosis} we
employed three independent methods. The first method used acid-fast stain
(auramine-rhodamine). The second method employed colony forming units
(CFU). After various time points of incubation, cells were lysed with 0.3%
saponin (Sigma) to release intracellular bacteria. At all time points, an
aliquot of unlysed, infected cells was harvested and counted. This allowed
an exact quantification of cells as well as the determination of cellular
viability by trypan blue exclusion. Recovery of cells was >80% in all
experiments, with cell viability regularly exceeding 90% of total cells.
Lysates of infected cells were resuspended vigorously, transferred into
spectrophotometer tubes, and sonicated at a preheated (37°C) water bath
sonicator (Elma, Singen, Germany) for 5 min. Aliquots of the sonicate were
diluted 10-fold in 7H9 medium. Four dilutions of each sample were plated in duplicates on
7H11 agar plates and incubated at 37°C and 5% CO$_2$ for 21 days. The third
method involved $[^{3}H]$uracil incorporation. Incorporation of $[^{3}H]$-labeled
uracil into the mycobacterial RNA was determined following the method
published by Rook and Rainbow (27) with several modifications. First, 1 x
10$^6$ infected DC were cultured in culture medium for 7 days before harvesting. The purity of the
macrophage population was confirmed by FACS staining (CD14$^+$, CD11b$^+$, CD19$^-$, CD56$^-$, CD3 $<$ 2%) and was above 98% in all
experiments.
\[ ^{3}H \text{H} \text{U} \text{r} \text{c} \text{i} \text{m} \text{p} \] incorporation was measured in a beta counter (Bertold, München, Germany). Background radioactivity in uninfected cells was below 500 cpm in all experiments. To document the suitability of our quantification methods to detect antimycobacterial activity, we performed some experiments in the presence of the mycobactericidal drug rifampicin (Sigma).

**Flow cytometry**

A total of \( 3 \times 10^5 \) cells were resuspended in 100 \( \mu l \) staining buffer (2% FCS, 1% NaN\(_{3}\), PBS without Mg\(^{2+}\)/Ca\(^{2+}\)) and incubated with unconjugated or conjugated Abs for 30 min on ice. Samples were washed twice in staining buffer and if necessary incubated for an additional 30 min on ice with goat anti mouse-FITC Abs (1:500). Cells were then fixed in 2% paraformaldehyde and stored at 4°C until analysis in a FACSscan flow cytometer. Data were analyzed using CellQuest software (Becton Dickinson).

**Mixed lymphocyte reaction**

DC were irradiated with 30 Gy (\(^{137}\)Cs source). Subsequently, graded numbers of these stimulator cells were mixed with a fixed amount of purified peripheral blood CD4\(^+\) cells (5 \( \times \) 10\(^5\)) and seeded into 96-well round-bottom tissue culture plates in a final volume of 200 \( \mu l \). CD4\(^+\) cells were enriched by indirect immunomagnetic depletion of cells expressing CD8, CD14, CD19, and CD56 (sheep anti mouse Dynabeads, Dynal, Oslo, Norway) from PBMC prepared by Ficoll-Hypaque density centrifugation. Purity, as determined by flow cytometry, proved to be \( >95\% \). Controls included DC alone, responder cells alone, and responder cells cultured in the presence of 100 U/ml IL-2. All cultures were set up as triplicates. After 5 days of culture, 0.5 \( \mu Cl \) \([^{3}H\]thymidine (Amersham-Pharmacia) was added to each well, and thymidine incorporation was measured 18 h later using a cell harvester and a betaplate counter.

**CD1-restricted cell lines**

CD1-restricted cell lines were generated as described earlier (28). DN7 was derived from a healthy, purified protein derivative-positive donor. DN7 is TCR\(^\beta\), CD4\(^+\), CD8\(^-\) and specifically recognizes purified lipoarabinomannan from *M. tuberculosis* in the context of the nonclassical MHC molecule CD1b. To measure the Ag-specific response, DN7 (1 \( \times \) 10\(^4\)) was incubated in the presence of IL-10-treated or control DC (1 \( \times \) 10\(^4\)) and lipoarabinomannan (1 \( \mu g/ml \)) for 3 days. The last 4 h of incubation were performed in the presence of 0.5 \( \mu Cl \) \([^{3}H\]thymidine. Incorporated radioactivity was measured after harvesting cells onto glass fiber filters in a beta counter.

**Statistical analysis**

Data are presented as mean value \( \pm \) SEM except where stated otherwise. Student’s *t* test was used to determine statistical significance between two differentially treated cultures. Differences were considered significant if *p* \( < 0.05 \).

**Results**

**Infection of human DC with virulent *M. tuberculosis***

To monitor the growth of *M. tuberculosis* in DC, it was necessary to determine the MOI, which allowed us to culture infected cells for at least 7 days. Therefore, we infected DC with an increasing MOI. Four hours later, extracellular bacteria were removed by differential centrifugation, and the number of infected cells was determined by acid-fast stain (Fig. 1A). MOIs of 2.5 or 12.5 resulted in a high infection efficiency of 62% and 93%, respectively. However, the high bacterial burden of the cells (Fig. 1B) resulted in cell death within 48 h (data not shown). A MOI of 0.5 resulted in infection of 24% \( \pm \) 0.4 of the cells, whereby each cell harbored an average of 2.2 \( \pm \) 0.3 bacteria. This infection rate did not result in rapid cell death and was chosen for studying the intracellular growth of *M. tuberculosis* in DC for 7 days. To ensure that we were studying the growth of intracellular, but not extracellular, mycobacteria, we double-stained infected DC with Abs directed against mycobacteria and DC. Bacteria were labeled with an anti-lipoarabinomannan Ab and a Texas Red-conjugated secondary Ab before infection of DC. Four hours after infection, DC were stained with an Ab recognizing the CD1a-FITC glycoprotein on the cell surface of DC. Confocal microscopy revealed that over

![FIGURE 1. Infection of DC with *M. tuberculosis*. Human DC were infected with an increasing number of *M. tuberculosis*. After 4 h of incubation, cells were fixed and stained with auramine-rhodamine. The graphs show the average results of eight independent experiments using DC derived from different donors. C, DC were infected with stained *M. tuberculosis* (anti-lipoarabinomannan, Texas-Red) and then labeled with anti CD1a (FITC). Cells were analyzed using confocal microscopy. The panel shows a representative overlay of the red (mycobacteria) and green (DC) fluorescence (1000-fold magnification).](http://www.jimmunol.org/}

[Image 308x158 to 536x731]
of each experiment, at least 300 cells were evaluated. The table presents the average number of infected cells and bacteria per cell (± SEM) and stained with auramine-rhodamine. The number of infected cells and the number of bacteria per infected cell were counted using a fluorescence microscope (594 nm). All samples were set up in duplicates. In each experiment, at least 300 cells were evaluated. The table presents the average number of infected cells and bacteria per cell (± SEM) calculated from seven independent experiments.

90% of the bacteria were localized intracellularly (Fig. 1C). To confirm the intracellular localization of M. tuberculosis, DC were harvested and centrifuged at 800 rpm. At this low speed, cells are preferentially spun down, while the majority of bacteria will remain in the supernatant. Plating of these supernatants revealed that <0.1% of the initial inoculum remained in the extracellular compartment (not shown).

**Growth of M. tuberculosis in human DC**

Using this model of low-dose infection (MOI 0.5), we found that after an initial quiescent phase of 24 h the number of M. tuberculosis increased 4-fold within 1 day and continued to multiply throughout the observation period (Fig. 2). Total growth of the bacteria was two orders of magnitude within 7 days, which is equivalent to an average generation time of M. tuberculosis in human DC of 26 h. The replication of bacteria resulted in an increased bacterial burden of individual cells, as well as a higher number of infected cells (Table I). Even though this finding indicates that some cells must have been lysed to allow spreading of the mycobacteria, the number of viable cells after 7 days of infection was above 90% of the initial inoculum (data not shown). To verify that we were not studying the growth of extracellular bacteria that had been released into the culture medium by dying cells, we centrifuged representative lysates at 800 rpm. These supernatants contained <0.5% of the total bacteria in the culture plate. Even though there was a considerable variability in the ability of the DC of individual donors to phagocytose M. tuberculosis (21–32%), the generation time remained similar. An extension of the observation period beyond 7 days was not informative as cell viability declined drastically.

**Influence of IFN-γ and TNF-α on the growth of M. tuberculosis**

Next, we designed experiments to investigate whether IFN-γ and TNF-α, alone or in combination, would be able to induce antimycobacterial activity in DC. While it is well established that IFN-γ activates antimycobacterial effector mechanisms in mice (29), evidence for comparable action on human macrophages is lacking (4, 5). Similarly, the role of TNF-α in the activation of human phagocytes is poorly defined, despite one study clearly showing an antimycobacterial effect of TNF-α on alveolar macrophages (30). Neither IFN-γ nor TNF-α (both at 10 ng/ml) reduced the metabolic activity of virulent M. tuberculosis in DC, as determined by incorporation of [3H]uracil (Fig. 3A). Higher cytokine concentrations up to 50 ng/ml also had no effect on bacterial growth (data not shown). TNF-α-treated cells showed a tendency to increase the bacterial burden. Even though this increase did not reach a significant level, it was reproducible in four of four independent experiments. Similarly, incubation of DC in the presence of a combination of IFN-γ and TNF-α had no effect on mycobacterial growth. As a control, we treated infected cells with the mycobacterial drug rifampicin, which reduced the uracil incorporation to background levels (<500 cpm) (Fig. 3A). This documents the suitability of our evaluation system to detect antimycobacterial activity.

**Influence of TGF-β, IL-4, and IL-10 on the growth of M. tuberculosis**

Prototypic cytokines involved in the down-regulation of the protective immune response against mycobacterial disease are TGF-β (31–33), IL-4 (34), and IL-10 (35–38). However, neither exogenous TGF-β nor IL-4 inhibited the metabolic activity of M. tuberculosis after an incubation period of 7 days in DC (Fig. 3B). To exclude that we had missed an early antimycobacterial effect of these cytokines, we also quantitated the bacterial load of cytokine-treated DC after 72 h of infection. No difference could be detected as determined by auramine-rhodamine stain (data not shown). Moreover, representative experiments revealed that replenishment of cytokines or Abs at day 3 did not alter the course of infection (data not shown). In contrast, IL-10 reduced the uracil uptake of M. tuberculosis from 16,265 cpm to 6,187 cpm by 62.5% (Fig. 3B). This unexpected finding was confirmed by determining the number of CFU of IL-10-treated and control cultures (Fig. 4A). A total of 1 ng/ml of IL-10 was already sufficient to decrease the mycobacterial growth by 43.5%. Increasing concentrations of IL-10 (10 ng/ml) enhanced the growth inhibition up to 65%. At 10 ng/ml, the activity of IL-10 reached a plateau and could not be augmented further (Fig. 4A). The reduced growth of M. tuberculosis in DC induced by IL-10 was not detectable 24 h after infection, ruling out an early antibactericidal effect associated with the uptake of the

![FIGURE 2. Growth of M. tuberculosis in DC, DC (5 × 10^5) were infected with M. tuberculosis at a MOI of 0.5. At the indicated time points, cells were lysed with saponin (0.3%) and plated on 7H11 agar plates in serial dilutions. All wells were set up in duplicates. The number of CFU was enumerated after 21 days and is given as total numbers of bacteria per well ± SD (calculated from the CFU for each dilution in duplicates). Data are representative for one experiment of 12 with similar results.](http://www.jimmunol.org/)

Table I. **Growth of virulent M. tuberculosis in human DC**

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected cells (%)</td>
<td>31 ± 4</td>
<td>36 ± 4</td>
<td>43 ± 7</td>
<td>55 ± 6</td>
<td>79 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>Bacteria/infected cell</td>
<td>2.3 ± 0.4</td>
<td>2.7 ± 0.3</td>
<td>5.9 ± 1.1</td>
<td>12.4 ± 2.7</td>
<td>24.2 ± 2.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Growth of M. tuberculosis in DC (5 × 10^5) were infected with M. tuberculosis at a fixed MOI of 0.5 on Permanox slides. At the indicated time points, cells were fixed and stained with auramine-rhodamine. The number of infected cells and the number of bacteria per infected cell were counted using a fluorescence microscope (594 nm). All samples were set up in duplicates. In each experiment, at least 300 cells were evaluated. The table presents the average number of infected cells and bacteria per cell (±SEM) calculated from seven independent experiments.*
cells were cultured in the presence of IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) alone or in combination. Additional samples were incubated with IFN-γ, TNF-α, and rifampicin for 24 h more in the presence of 3 μCi [3H]uracil. All wells were set up in triplicates. The graph presents the average of four experiments using DC from different donors. Data are shown as cpm ± SEM.

**FIGURE 3.** Influence of cytokines on the growth of *M. tuberculosis*. DC 
(5 × 10^5) were infected with *M. tuberculosis* at a MOI of 0.5. A, Infected cells were cultured in the presence of IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) alone or in combination. Additional samples were incubated with rifampicin (2 μg/ml). After 7 days, cells were lysed and incubated for an additional 24 h in the presence of 3 μCi [3H]uracil. All wells were set up in triplicates. The graph presents the average of four experiments using DC from different donors. Data are shown as cpm ± SEM. B, IL-4, TGF-β, or IL-10 (all at 10 ng/ml) were present during the 7-day culture period before determining the uptake of [3H]uracil. All wells were set up in triplicates. Starred bars demonstrate statistical significance (p < 0.05) between cytokine-treated and untreated cultures.

Influence of cytokines on the growth of *M. tuberculosis* in human DC. Because IFN-γ is also secreted by DC infected with live *M. tuberculosis* (13), we considered the possibility that neutralization of endogenous IL-10 would increase mycobacterial survival. However, no difference in mycobacterial growth was observed compared with the uninfected controls (Fig. 4B). This result is in agreement with our failure to detect IL-10 in the supernatant of infected DC (<20 pg/ml, data not shown). To our knowledge, only one study has shown the secretion of IL-10 by *M. tuberculosis*-infected immature DC (13). Using a MOI of 5–10 (as opposed to the MOI of 0.5 used in our experiments), they found 100 pg/ml of IL-10 in the supernatant. These values are one order of magnitude lower than the amounts needed to observe an antimycobacterial effect in our study.

**IL-10 converts DC into macrophage-like cells**

To unmask the mechanism of the IL-10-induced growth inhibition, we considered the possibility that IL-10 influences the phenotypic and functional maturity of monocyte-derived DC. We incubated uninfected DC, generated as described above, for an additional 48 h in the presence of IL-10 or medium alone (Fig. 5A). This resulted in a marked up-regulation of the monocyte marker CD14 (mean fluorescence intensity 345 vs 1987 after IL-10 treatment). The CD1a molecule, which is highly expressed on the cell surface of immature DC, almost disappeared from the cell surface (Fig. 5A). The functional impact of the down-regulation of CD1 was demonstrated by the failure of these cells to present lipoprotein-mannan to a CD1b-restricted T cell line (Fig. 5B). To further characterize the functional impact of IL-10 treatment on immature DC, we measured the ability to induce a mixed lymphocyte reaction. Incorporation of [3H]thymidine by heterologous, CD4^+ T cells was significantly lower when IL-10-treated cells were used as APC (Fig. 5C). Because DC lose the ability to phagocytose soluble and particulate Ags during their maturation from monocytes (39, 40), we asked whether IL-10 treatment of immature DC would reverse this effect and increase the ability to phagocytose virulent *M. tuberculosis*. IL-10 treatment increased the phagocytic activity of DC in a dose-dependent manner from 32 ± 2% in untreated cells up to nearly 80 ± 4% by treatment with 10 ng/ml of IL-10 (Fig. 5D). In summary, these experiments demonstrate that IL-10 converts immature DC into macrophage-like cells according to phenotypic and functional criteria. Therefore, the reduction of mycobacterial growth in IL-10-treated immature DC may be the result of the modulation of the maturity of the host cell, rather than a consequence of antimycobacterial effector mechanisms in DC.

**Effect of IL-10 on mycobacterial growth and the phenotype of macrophages**

To investigate whether the described effects of IL-10 are unique to immature DC or represent a general phenomenon, we examined the growth of *M. tuberculosis* in human macrophages. Macrophages were derived from peripheral blood monocytes and cultured for 7 days before infection with *M. tuberculosis* with a MOI of 0.5. IL-10 was added after the end of the pulse infection at various concentrations (ranging from 1 ng/ml to 50 ng/ml), and mycobacterial growth was determined after 7 days of culture. We found no influence on mycobacterial growth at all concentrations tested (Fig. 6A). Similarly, treatment of macrophages with IL-10 (10 ng/ml) for 48 h did not modulate the expression of CD14 and CD1a on the cell surface (Fig. 6B) as opposed to its effect on DC (Fig. 5A). To verify the biologic activity of IL-10 on macrophages, we also measured MHC class II expression, which is known to be down-regulated by IL-10 (41). As expected, expression of MHC class II molecules on the cell surface was down-regulated by IL-10 (mean fluorescence intensity of 199 vs 49 after treatment), whereas it had no effect on MHC class II expression on immature DC (Fig. 5A). Taken together, these data demonstrate that the induction of antimycobacterial activity and regulation of CD14/CD1a by IL-10
is specific for immature DC and is not a general phenomenon of host cells for intracellular pathogens.

**Macrophages are superior to DC in inhibiting the growth of M. tuberculosis**

Our previous experiments had demonstrated that IL-10 converts immature DC into cells resembling macrophages. These macrophage-like cells might be more competent in combating an infection with intracellular pathogens. To test this hypothesis, we compared the intracellular growth of *M. tuberculosis* in macrophages and DC derived from the same donor. In four independent experiments using different donors, we found that mycobacterial growth was significantly lower in macrophages than in DC after 7 days of incubation. Using an identical MOI

![Graph A: Colony forming units (x10^6) vs. IL-10 (ng/ml)]

![Graph B: Colony forming units (x10^6) vs. time after infection (days)]

![Image C: Untreated DC vs. IL-10 treated DC](untreated DC vs. IL-10 treated DC)

**FIGURE 4.** IL-10 inhibits the growth of *M. tuberculosis* in DC. A, DC (5 × 10^5) were infected with *M. tuberculosis* at a MOI of 0.5. After 7 days of incubation in the presence of the indicated concentrations of IL-10, cells were lysed and plated on 7H11 agar plates in serial dilutions. All samples were set up in duplicates. The number of CFU was enumerated after 21 days and is given as the average numbers of CFU per well ± SEM (calculated from the CFU of three independent experiments). Starred bars demonstrate statistical significance (*, p < 0.05; **, p < 0.01) between IL-10-treated and the control culture. B, DC were infected with *M. tuberculosis* at a MOI of 0.5. After 1, 3, and 7 days of incubation in the presence of IL-10 (10 ng/ml) or anti IL-10 (20 μg/ml), cell lysates were plated on 7H11 agar plates in serial dilutions. All samples were set up in duplicates. The graph shows the average result of four experiments with different donors ± SEM. The star indicates statistical significance (p < 0.05) between IL-10-treated and untreated cultures. C, DC were cultured on Permanox chamber slides and infected with *M. tuberculosis* at a MOI of 0.5. After 7 days of incubation in the presence of IL-10 (10 ng/ml) or medium alone, infected cells were fixed and stained with auramine-rhodamine. Intracellular mycobacteria were detected by confocal laser-microscopy at a wavelength of 594 nm (upper panels) or bright field illumination (lower panels). All samples for confocal analysis were set up in quadruplicates, and the pictures present a representative sector of a control (left panels) and IL-10-treated (right panels) culture. Magnification, ×1000.
(0.5), we found that the initial inoculum was higher in macrophages than in DC (data not shown), consistent with our previous data (Fig. 5D). After 3 days of infection, and even more significantly after 7 days of infection, mycobacterial multiplication was lower in macrophages as compared with DC. Within the 7-day observation period, the bacterial burden in DC increased 72-fold in DC and only 24-fold in macrophages. This results in a doubling time of M. tuberculosis in DC of 24 h and for bacteria residing within macrophages of 42 h (Fig. 7). To rule out that the difference in the initial inoculum influences the generation time of mycobacteria, we adjusted the MOI in macrophages such that the infection rate was similar to DC (23%). This had no significant impact on the intracellular growth of M. tuberculosis in macrophages (data not shown).

Table II. Growth of M. tuberculosis in IL-10-treated DC

<table>
<thead>
<tr>
<th>IL-10 (ng/ml)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected cells (%)</td>
<td>86 ± 4</td>
<td>46 ± 3</td>
<td>31 ± 2</td>
<td>16 ± 3</td>
<td>21 ± 3</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>Bacteria/infected cell</td>
<td>ND</td>
<td>9.2 ± 1.9</td>
<td>4.1 ± 1.1</td>
<td>2.3 ± 0.5</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.6</td>
</tr>
</tbody>
</table>

*Growth of M. tuberculosis in DC. DC (5 × 10⁵) were infected with M. tuberculosis at a MOI of 0.5 on Permanox slides. After 7 days of incubation, the cells were fixed and stained with auramine-rhodamine. The number of infected cells and the number of bacteria per infected cell were analyzed using a confocal laser microscope. All samples were set up in duplicates. For each sample, at least 300 cells were evaluated. The table presents the average number of infected cells and bacteria per cell (±SEM) calculated from five independent experiments.

FIGURE 5. IL-10 converts immature DC into macrophage-like cells. A. Adherent monocytes were cultured in the presence of GM-CSF (1000 U/ml) and IL-4 (1000 U/ml) for 7 days. Cultures were then supplemented with IL-10 (10 ng/ml) and incubated for additional 48 h. Nonadherent and adherent cells were harvested and pooled for further analysis. Cells were stained using FITC-conjugated Abs directed against CD14, CD1a, or MHC class II. A total of 10,000 cells were analyzed per sample. Data are representative of five independent experiments. B. A total of 1 × 10⁴ T cells of DN7 were coincubated with 2 × 10⁴ DC (IL-10-treated or control) for 72 h in the presence or absence of lipoarabinomannan (LAM, 2 μg/ml) and pulsed with 0.5 μCi [3H]thymidine for the final 4 h. All wells were set up in triplicates. The data show the average x-fold thymidine uptake ± SEM of LAM-treated cells as compared with unstimulated control cultures. Background levels in unstimulated cultures were below 1000 cpm in all four experiments shown. C. Graded numbers of irradiated, IL-10-treated, and control DC were coincubated with purified heterologous CD4⁺ T cells (5 × 10⁴). Cells were incubated for 6 days, and [3H]thymidine was present during the final 18 h of culture. All samples were performed in triplicates. Data present the average thymidine uptake calculated from four independent experiments ± SEM. The star indicates statistical significance (*, p < 0.05) between IL-10-treated and untreated DC. D. Immature DC were cultured for 48 h in the presence of rising concentrations of IL-10 as indicated. Cells were then harvested, plated on Permanox chamber slides (2 × 10⁶/chamber), and infected with M. tuberculosis at a MOI of 0.5. After 4 h of incubation, cells were fixed and stained with auramine-rhodamine. The stained cells were examined under a fluorescence microscope (wavelength, 594 nm), and the percentage of infected cells was determined. For each MOI, at least 300 cells were examined for the presence of intracellular acid-fast rods. The graph shows the average number of infected cells calculated from five independent experiments using DC derived from different donors (% infected cells ± SEM). Starred bars demonstrate statistical significance (*, p < 0.05; **, p < 0.01) between IL-10-treated and control cultures.
not capable of limiting the intracellular growth of human pathogen. We demonstrate that immature human DC are primary T cell response (39, 40), this study is the first to investi-
has become a paradigm that DC are indispensible for initiating a role of DC in immunity to intracellular pathogens. While it Our study presents a novel approach to extend the understanding Discussion

FIGURE 6. Influence of IL-10 on the growth and phenotype of macrophages. A, Macrophages (5 \times 10^5) were infected with M. tuberculosis at a MOI of 0.5. IL-10 was added at the indicated concentrations after 4 h pulse infection. After 7 days, cells were lysed and plated on 7H11 agar plates. All samples were set up in duplicates. The number of CFU is presented as the average numbers of CFU per well \pm SEM (calculated from the CFU for three independent experiments). B, Macrophages were incubated with IL-10 (10 ng/ml) and incubated for 48 h. Cells were stained using FITC-conjugated Abs directed against CD14, CD1a, or MHC class II. A total of 10,000 cells were analyzed per sample. Data are representative for three independent experiments.

FIGURE 7. Macrophages and immature DC (1 \times 10^6 per well) from identical donors were infected with M. tuberculosis (MOI 0.5). The initial number of intracellular bacteria was determined 4 h after infection and was 1.25 \times 10^9 and 2.05 \times 10^9 for DC and macrophages, respectively. This bacterial load was used for the calculation of x-fold proliferation of M. tuberculosis after 1, 3, and 7 days of infection. The bacterial load was determined by plating lysates of the infected cells on 7H11 agar and enumerating the number of CFU after 21 days. All wells were set up in duplicates and plated in four dilutions. The data are presented as average of the x-fold proliferation \pm SEM calculated from the single counts of each dilution of four independent experiments. The star indicates a significant difference (p < 0.05) between the mycobacterial growth in macrophages and DC.

vent Ag presentation of lipid Ags to cytolytic T cells by down-regulating one of the Ag-presenting molecules, i.e., CD1 (42). We extend these findings by showing that invasion of DC by virulent bacteria may be beneficial for the pathogen as it can multiply liberally. Therefore, invasion of DC may be advantageous for intra-cellular organisms allowing their unlimited multiplication and spreading. In contrast to the phagocytosis of soluble microbial Ag, which leads to the maturation of DC and subsequent activation of the specific immune system, uptake of viable pathogens might be harmful for the host. Therefore, the role of DC in the local immune response might be both harmful or beneficial to the host, depending on whether soluble Ags or live microbes are taken up.

M. tuberculosis infects humans primarily by the respiratory route. The majority of cells present in the alveolar spaces are alveolar macrophages that phagocyte the microbial invader (6). However, alveolar macrophages are not efficient in inhibiting the growth of intracellular pathogens and have even been shown to depress the protective immune response of Ag-specific T cells (43). Another cell type present in the airway epithelium are DC (7). Intracellular bacteria that can invade DC comprise relevant human pathogens (12–20). Therefore, it is becoming increasingly important to learn more about the intracellular fate of live microorganisms residing within DC. Human DC infected in vitro with M. tuberculosis undergo a direct activation and maturation that presumably enhance their efficacy at stimulating T cells (13). In contrast, M. tuberculosis has evolved evasion mechanisms to pre-

Intracellular bacteria that can invade DC comprise relevant human pathogens (12–20). Therefore, it is becoming increasingly important to learn more about the intracellular fate of live microorganisms residing within DC. Human DC infected in vitro with M. tuberculosis undergo a direct activation and maturation that presumably enhance their efficacy at stimulating T cells (13). In contrast, M. tuberculosis has evolved evasion mechanisms to pre-

Intracellular bacteria that can invade DC comprise relevant human pathogens (12–20). Therefore, it is becoming increasingly important to learn more about the intracellular fate of live microorganisms residing within DC. Human DC infected in vitro with M. tuberculosis undergo a direct activation and maturation that presumably enhance their efficacy at stimulating T cells (13). In contrast, M. tuberculosis has evolved evasion mechanisms to pre-

Intracellular bacteria that can invade DC comprise relevant human pathogens (12–20). Therefore, it is becoming increasingly important to learn more about the intracellular fate of live microorganisms residing within DC. Human DC infected in vitro with M. tuberculosis undergo a direct activation and maturation that presumably enhance their efficacy at stimulating T cells (13). In contrast, M. tuberculosis has evolved evasion mechanisms to pre-

Intracellular bacteria that can invade DC comprise relevant human pathogens (12–20). Therefore, it is becoming increasingly important to learn more about the intracellular fate of live microorganisms residing within DC. Human DC infected in vitro with M. tuberculosis undergo a direct activation and maturation that presumably enhance their efficacy at stimulating T cells (13). In contrast, M. tuberculosis has evolved evasion mechanisms to pre-
IL-10 LIMITS THE GROWTH OF M. tuberculosis IN DENDRITIC CELLS

overwhelming bacterial burden. These apoptotic bodies could then be taken up by DC, which present mycobacterial Ags to T cells. While direct evidence for the uptake of regurgitated mycobacterial products by DC is lacking (44), DC that have taken up Ags derived from apoptotic bodies have been shown to activate CD8+ cytolytic T cells (45). CD8+ T cells have been suggested to play a special role in the human immune response to M. tuberculosis by injecting anti-mycobacterial effector molecules such as granulysin into the target cell (46). The special role of DC in Ag presentation is underscored by their unique ability to present nonprotein Ags to T cells via the nonclassical MHC molecules CD1a, -b, and -c (47). Because our experiments demonstrate that DC are poor antibacterial effector cells (Figs. 2 and 7) and are clearly inferior to macrophages in this regard, their prominent function is more likely to link the innate and acquired immune response by recruiting and activating Ag-specific T cells.

IL-10 was first detected based on its cytokine synthesis inhibitory activity mainly on macrophages (48–50). Recently, evidence is accumulating that DC are another major target for the action of this immunosuppressive cytokine. IL-10 was reported to inhibit the Ag-presenting capacity of DC (51–55), to reduce the expression of Ag-presenting and costimulatory molecules (56–59), and to interfere with the maturation of monocytes to DC (60–62). Taken together, these studies suggest an overall picture in which IL-10 prevents the differentiation of monocytes to mature DC but promotes their maturation to macrophages (63). Recent findings demonstrate that maturation of monocytes to DC also occurs in vivo (64, 65). These findings point out that the development of monocyte cells into either mature tissue-macrophages or DC depends on the local microenvironment. The opposite development of immature DC into macrophages as shown here in an in vitro culture system has not been formally proven in vivo. However, the studies discussed above demonstrate the potential of immature cells to develop differentially in vivo. This may serve to pave the way for the maturation of cells optimally equipped to meet the functional requirements of the local immune response. Specifically, in the case of a tuberculous granuloma, the local cytokine microenvironment may be dominated by IL-10 in certain instances (36), thereby supporting the development of macrophage-like cells. These will then complement effector mechanisms of the protective cellular immune response and contribute to the eradication of M. tuberculosis.

One mechanism of infected cells to eliminate intracellular pathogens is to undergo apoptosis, thereby exposing the microbes to the extracellular environment. IL-10 has been shown to inhibit apoptosis of cells infected with intracellular bacteria including mycobacteria (66, 67). However, we did not observe apoptosis in untreated or IL-10-treated immature DC as determined by annexin V staining (data not shown). The failure to detect apoptotic cells was most likely a consequence of the low MOI, which was chosen to allow a 7-day observation period of mycobacterial growth. Also, if IL-10 would inhibit apoptosis of infected DC, bacterial growth would more likely be increased, rather than inhibited as observed in this study. Therefore, growth inhibition of mycobacteria in immature DC by IL-10 is unlikely to be mediated by the modulation of apoptosis.

In vivo studies revealed that IL-10-expressing cells tend to accumulate in patients suffering from an unfavorable outcome of disease (35, 68). However, the data from murine studies argue against a critical, nonredundant role of IL-10 in immunity to tuberculosis because IL-10-deficient mice are not or are only partially protected from tuberculosis (38, 69).

This study exemplifies the versatility of the immune system with an armamentarium of effector cells, each having specific and specialized functions in immunity to microorganisms. In the setting of a local cellular immune response, as is typical for a tuberculous granuloma, the differential effects of IL-10 on macrophages and DC may contribute to the fine-tuned balance that must provide protection from the pathogen, as well as limitation of excessive tissue destruction.

Acknowledgments

We acknowledge the expert technical assistance of Kirstin Castiglione and Nives Schwender. We thank Dr. Heinrich Körner for critical reading of the manuscript, and Dr. Klaus Schröppel for helping us with the annexin staining.

References


