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Down-Regulation of CD1 on Antigen-Presenting Cells by Infection with *Mycobacterium tuberculosis*[1]

Steffen Stenger,[2] Kayvan R. Niazi, and Robert L. Modlin[3]

Intracellular pathogens have developed efficient evasion strategies to survive the defenses of the host immune system. In this study, we describe a new escape mechanism utilized by *Mycobacterium tuberculosis* that involves the down-regulation of the Ag-presenting molecule CD1 from the cell surface of CD1$^+$ APCs. The loss of CD1 from the cell surface is associated with a complete inhibition of the ability of the infected cells to present Ag to CD1-restricted T cells. The down-regulation of Ag-presenting molecules on CD1$^+$ APC by infection with *M. tuberculosis* is unique for CD1, since the expression of the classical Ag-presenting molecules MHC class I and MHC class II is not influenced. Our data show that efficient down-regulation of CD1 requires infection of the cells with live mycobacteria, since heat killing of the bacteria completely abrogates the effect. The observed down-regulation is not due to the secretion of cytokines or other host- or pathogen-derived factors. Investigation of upstream events responsible for the down-regulation of CD1 revealed that infection with live *M. tuberculosis* decreased the steady state CD1-mRNA levels. This study introduces a novel evasion mechanism of *M. tuberculosis* that could contribute to persistence of intracellular infection by avoiding immune recognition. *The Journal of Immunology,* 1998, 161: 3582–3588.

Successful elimination of the intracellular pathogen *Mycobacterium tuberculosis* depends mainly on the efficient interplay of infected macrophages with Ag-specific T cells. In 90 to 95% of persons who are exposed to *M. tuberculosis*, the interactions of the different components of the cellular immune response prevent clinically overt disease (1). However, even if an inapparent tuberculous infection heals, bacilli may persist in a dormant state (2). Latency of infection can last lifelong or lead to active disease when the efficiency of the immune system is impaired (e.g., old age (3), corticosteroids (4), or AIDS (5, 6)).

Macrophages are critical cells in the pathogenesis of tuberculosis infection. Initially, the macrophage serves as the host cell for the pathogen. However, upon receipt of appropriate activation signals from Ag-specific T cells, the macrophage converts into a powerful effector cell with the ability to kill its invader. A central role for macrophages is the presentation of Ags to T cells. Classically, macrophages present peptides to T cells in the context of MHC class I and II molecules. Recently, a new Ag presentation pathway involving the CD1 proteins has been identified. These polypeptides are expressed constitutively on professional APCs and can be induced on peripheral blood monocytes by treatment with granulo-cyte-macrophage CSF (GM-CSF)[4] and IL-4 (7, 8). A unique feature of CD1-Ag presentation is the presentation of lipid and lipoglycan Ags to T cells. CD1-restricted T cells can contribute to protective immunity by the production of high levels of IFN-γ (9) and lysis of macrophages infected with virulent *M. tuberculosis*, leading to the killing of intracellular bacteria (10).

We considered the possibility that the CD1-Ag presentation pathway system might be an attractive target for *M. tuberculosis* in its attempt to escape recognition and destruction by the immune system. We therefore infected CD1$^+$ APC with a virulent strain of *M. tuberculosis* and investigated the impact of the infection on CD1-Ag presentation. We found that infection with *M. tuberculosis* leads to the down-regulation of surface CD1 expression at the mRNA level. The resulting inability of infected cells to present Ag to CD1-restricted T cells demonstrates a novel evasion strategy utilized by an intracellular pathogen to circumvent immune recognition.

Materials and Methods

Cell culture reagents

T cells were cultured in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 0.1 mM sodium pyruvate, 2 mM glutamine (Sigma, St. Louis, MO), 8% FCS (HyClone Laboratories, Logan, UT), and 2% heat-inactivated human serum. Experiments only involving APCs were performed in 10% FCS.

Cytokines and Abs

The following cytokines and Abs were used: recombinant human (rhu) IL-6 (R&D Systems, Minneapolis, MN), rhuIL-12 (gift of M. Gately, Roche, Nutley, NJ), rhuIL-10 (gift of K. Moore, DNAX, Palo Alto, CA), rhuTNF-α (Endogen, Woburn, MA), purified TGF-β (R&D Systems, Minneapolis, MN), anti-IL-6 (Biosource International, Camarillo, CA; clone B-E8), anti-IL-12 (Roche; clone 2-4A1), anti-IL-10 (PharMingen, San Diego, CA; clone JES3-9D7), anti-TGF-β (Genzyme, Cambridge, MA), anti-TNF-α (polyclonal), IgG1 FITC (Caltag, Burlingame, CA), IgG1 (Sigma), and IgG2a (Sigma).

Growth of *M. tuberculosis*

M. tuberculosis (virulent strain H37Rv) was grown in suspension with constant, gentle rotation in roller bottles containing Middlebrook 7H9 broth

[4] Abbreviations used in this paper: GM-CSF, granulocyte-macrophage CSF; MOI, multiplicity of infection; PE, phycoerythrin; rhu, recombinant human.

(Difco, Detroit, MI) supplemented with glycerol, 0.05% Tween-80 (Sigma), and 10% Middlebrook OADC enrichment (Difco). Aliquots from logarithmically growing cultures were frozen in PBS containing 10% glycerol, and representative vials were thawed and enumerated for viable CFU on Middlebrook 7H11 plates. Comparison of microscope counts of mycobacteria and their growth on agar plates revealed a viability of the bacteria above 90%. Since clumping of mycobacteria is a common problem that can influence the validity and reproducibility of the experiments, we undertook several precautions to minimize clumps: 1) Culture conditions were chosen to support the growth of single cell suspensions. 2) Before in vitro infection, *M. tuberculosis* bacilli were sonicated to disrupt small aggregates of bacteria. 3) The multiplicity of infection (MOI) was selected such that there were only two to three bacilli per APC. 4) At the conclusion of every assay, an aliquot of infected cells was stained with auramine rhodamine to confirm the absence of any clumps.

Infection of CD1$^+$ APC

PBMCs from healthy donors were treated with GM-CSF (200 U/ml) and IL-4 (100 U/ml) for 3 days. Adherent cells were detached by treatment with 1 mM EDTA (Sigma) and replated in six-well plates at a density of 3×10^6 cells/well. Monolayers were infected with single cell suspensions of *M. tuberculosis*. In some experiments, bacteria were heat killed before infection by incubation at 80°C for 30 min. This treatment killed all mycobacteria, as determined by CFU count. The gross morphology of the heat-killed bacteria was not altered according to auramine-rhodamine and Ziehl-Neelsen staining. After 4-h incubation, extracellular bacteria were removed by extensive washing. The efficiency of infection was quantitated in each experiment by staining of parallel cultures with auramine-rhodamine. In all experiments, the efficiency of infection was 3.47 ± 0.27 bacteria/cell, whereby $81 \pm 4\%$ of the cells were infected. No differences were observed in cultures infected with live or heat-killed *M. tuberculosis*. The microscopic evaluation of infected macrophages under the fluorescence microscope confirmed the absence of any mycobacterial aggregates. Cell viability of infected APC was determined by measuring the enzymatic activity of lactate dehydrogenase in the culture supernatant (CytoTox 96; Promega, Madison, WI).

FACS analysis

CD1$^+$ APCs were pulse infected for 4 h, as described above, and incubated for different time intervals. In some experiments, recombinant cytokines or Abs to cytokines were added during the incubation period. At indicated time points, cells were harvested by treatment with 1 mM EDTA and washed. A total of 3×10^5 cells was resuspended in 100 μl FACS buffer (2% FCS, 1% NaN$_3$, PBS without Mg^{2+}/Ca^{2+}) and incubated with unconjugated (MHC class I, clone W6/32, and MHC class II, clone L243, both obtained from American Type Culture Collection (ATCC), Manassas, MD; IgG1 control, obtained from Sigma, final concentration 2.5 μg/ml) or conjugated Abs (anti-CD1a FITC, clone OKT6 obtained from ATCC; anti-CD1b FITC, clone WM-25, Serotec, Raleigh, NC; IgG1 FITC, IgG2a FITC, HLA-DR FITC, all obtained from Caltag; CD14 PE, B7.1 PE, B7.2 PE, IgG PE, all obtained from Becton Dickinson, Mountain View, CA; 3 μl/sample) for 30 min on ice. Samples were washed twice in FACS buffer, and if necessary, an incubation for additional 30 min on ice with goat anti-mouse FITC Abs (Caltag; 1:250) followed. Cells were then fixed in 2% paraformaldehyde and stored at 4°C until analysis on a FACScan flow cytometer, and data were analyzed using Winmdi software.

Transwell experiment

CD1$^+$ APCs were infected and harvested as described above. A total of 1×10^6 infected cells was plated in the upper chamber of a transfer system (Costar, Cambridge, MA; six-well plates), which is separated from the lower chamber by a membrane permeable only for particles smaller than 0.4 μm. The diameter of the pores allows cytokines and secreted mycobacterial Ags, but not whole bacteria to pass. In the lower chamber, 3×10^6 uninfected CD1$^+$ APC were cultured. After 48 h, the cells in the lower chamber were detached and FACS staining was performed, as described above.

IFN-γ ELISA

CD1$^+$ APC were infected with live or pulsed with dead *M. tuberculosis*, as described above. Immediately after the end of the 4-h pulse infection or after an additional culture of 48 h, cells were detached by treatment with 1 mM EDTA. To determine their capacity to present Ag of the phagocytosed mycobacteria, 1×10^4 cells were replated in triplicates in 96-well round-bottom plates. T cells (1×10^4) were added (final volume 200 μl),

and after a coincubation of 16 h, supernatants were assayed for IFN-γ content in a capture ELISA using two distinct IFN-γ Abs (Endogen). Ninety-six-well plates were coated with capture Abs, blocked, and incubated with IFN-γ standards or the culture supernatants. The captured IFN-γ was detected with a biotinylated anti-IFN-γ detection Ab, followed by incubation with streptavidin-peroxidase (Sigma) and the chromogenic peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The absorbance at 405 nm was measured with an ELISA plate reader, and the IFN-γ concentrations were determined from the standard curve.

RT-PCR and Southern blot analysis

The synthesis of cDNA from 2×10^6 CD1$^+$ APC was performed as described (11). The cDNA was amplified using the following oligonucleotide pairs: 1) CD1a, ATGCTGTTTTTGCTACTTCCATTGTTA/CGTGGGCAGGTGTC ACTGAGAAG; 2) CD1b, ATGCTGCTGCTGCCATTTCAACTGTTA/GG GCAGGTTTCATAGAGGAGAATTC; and 3) CD1c, ATGCTGTTTCTGC AGTTTCTGCTGCTA/CTGTTTCTGTGACGCCTTCATACTG and β-actin (11). The CD1 cDNA products were amplified using 30 cycles (1′ 94°C/2′ 55°C/2′ 72°C) of time/temperature conditions. The β-actin cDNA product was amplified using 30 cycles (1 min at 94°C, 2 min at 60°C, and 2 min at 72°C) conditions. PCR products were separated on a 2% agarose gel, blotted, and probed (11) with the respective γ-^{32}P end-labeled oligonucleotides (CD1a, CTGGAAGGCATGTTCACTGT; CD1b, CAGCTCACAGCCTCCTGT CACCTG; CD1c, GCAGCTCACAGCCCGCTTTCACCTG; and β-actin) (11). Following this procedure, the filters were exposed to autoradiography films for analysis.

Results

Down-regulation of CD1 expression in CD1$^+$ APCs

To investigate whether infection with *M. tuberculosis* influences the expression of the Ag-presenting molecule CD1, we developed an experimental system to infect adherent CD1$^+$ cells with a virulent strain of *M. tuberculosis*. Total PBMC, which do not constitutively express CD1, were treated with a combination of GM-CSF and IL-4. After 72 to 96 h, adherent aggregates of large cells with prominent cytoplasmic processes had developed. Adherent cells were isolated by discarding the nonadherent fraction and detaching the remaining cells by Ca^{2+} chelation. FACS analysis showed expression of high levels of CD1a, CD1b, CD1c, B7.2, and HLA-DR, as well as expression of B7.1 and CD4 (Fig. 1). In contrast, staining for the monocyte/macrophage marker CD14 (Fig. 1), as well as staining for T cells (CD3), B cells (CD19), and NK cells (CD56) was negative, indicating the purity of the cultures. This argues against the possibility that cytokine production by nonadherent lymphocytes influences our results. This staining pattern in conjunction with the morphologic appearance indicates that the cells used in our experiments are likely to belong to the dendritic cell lineage (7).

CD1$^+$ APC were infected with *M. tuberculosis* at an MOI of 10:1 for 4 h. This protocol resulted in an infection efficiency of 83% with approximately three bacteria per cell. The level of CD1b expression on the cell surface of infected CD1$^+$ APC was determined at different time points after infection by FACS analysis. After 4 h of infection, no significant differences were observed between the infected cells and the uninfected controls (Fig. 2). However, after 24 h, the intensity of the CD1a, CD1b, and CD1c staining on the cell surface started to decline and was not detectable after 48 h. Uninfected CD1$^+$ APC maintained the high level of CD1 expression throughout the duration of the experiment (Fig. 2).

Down-regulation of CD1 could reflect a nonspecific inhibition of Ag presentation molecules on infected APC. We therefore investigated the expression of the classical Ag-presenting molecules MHC class I or class II in the same experiment. Expression of both class I and class II molecules was not affected by infection with live *M. tuberculosis* at any time point (Fig. 2). At 48 h of infection, CD1$^+$ APC harbored approximately seven to eight bacteria and

FIGURE 1. Total PBMC were treated with GM-CSF (200 U/ml) and IL-4 (100 U/ml) for 3 days. The expression of cell surface markers (——) was analyzed on the adherent fraction (CD1$^+$ APC) by FACS using directly conjugated Abs or an isotype control (---). The figure shows one representative experiment of six.
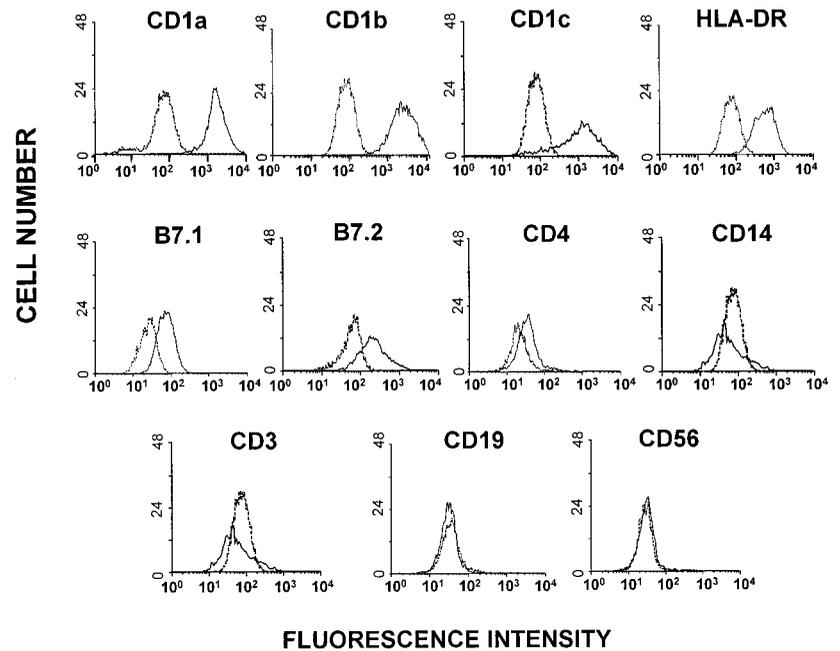
more than 95% of the cells were infected. Despite the high bacterial burden, the viability of the cells was above 80%, as determined by release of lactate dehydrogenase in the supernatant.

We confirmed our initial finding by studying the CD1 expression on infected CD1$^+$ APC of nine different donors in independent experiments. In addition to infecting the cells with viable bacteria, the cells of four donors were pulsed with heat-killed bacteria in parallel wells. All donors tested showed a significant down-

regulation of CD1 in response to infection with live *M. tuberculosis* after 48 h (Fig. 3, *left panel*). In contrast, incubation with dead mycobacteria had no effect on CD1 expression (Fig. 3, *right panel*). Even though phagocytosis of live and dead mycobacteria was equally efficient, the uptake and harboring of the bacteria alone were not sufficient to induce down-regulation of CD1. Down-regulation of the CD1 molecules from the cell surface of cells infected with viable *M. tuberculosis* was not confined to
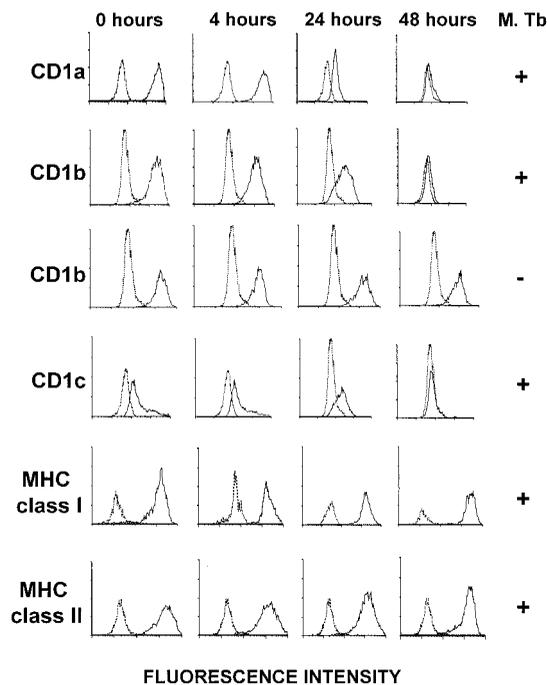
FIGURE 2. CD1b expression on CD1$^+$ APC infected with live *M. tuberculosis*. CD1$^+$ APC were infected with live *M. tuberculosis* at an MOI of 10. At indicated time points, cells were detached and the expression of cell surface markers was analyzed by flow cytometry using FITC-conjugated Abs (——) or an isotype control mAb (---). The figure shows the results from one representative experiment of three.
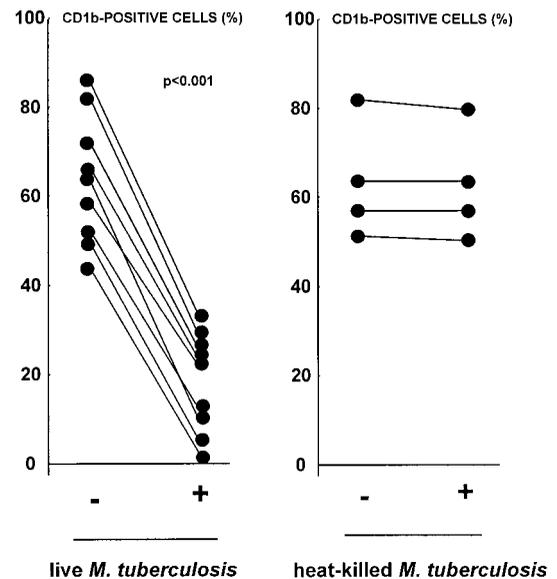
FIGURE 3. CD1b expression on CD1$^+$ APC infected with live or dead *M. tuberculosis*. PBMC from different donors were incubated for 3 days in GM-CSF and IL-4, and the adherent cells were infected with live (*left panel*) or heat-killed (*right panel*) *M. tuberculosis* at an MOI of 10. After 48 h, cells were detached and expression of CD1b was analyzed by flow cytometry with anti-CD1b FITC mAb or an isotype control mAb. Results are expressed as percentage of positive cells as compared with the isotype control. The *p* value was calculated according to the Signed rank test for paired samples.

T cells added
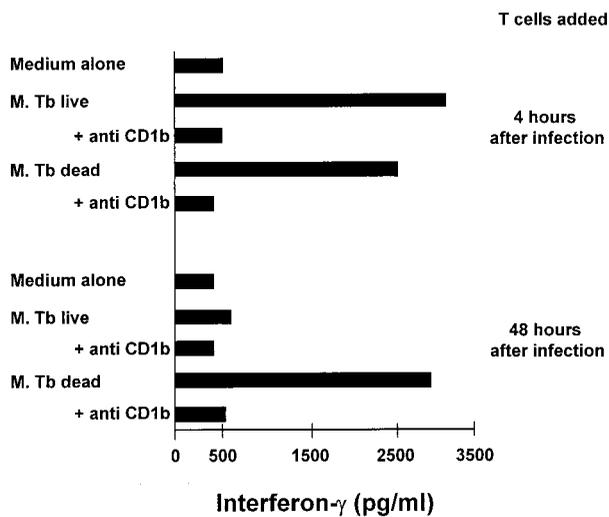
FIGURE 4. Ag-presenting function of CD1$^+$ APC infected with *M. tuberculosis*. Adherent CD1$^+$ APC were infected with live or heat-killed *M. tuberculosis* at an MOI of 10. Uninfected controls were cultured identically. After 4 and 48 h, cells were harvested and replated at a density of 3 × 10^4/200 μl in a 96-well plate. Infected cells were then coincubated with 1 × 10^4 DN.PT for 16 h, and the IFN-γ release in the supernatants was determined by ELISA. In indicated wells, cells were incubated in the presence of a blocking Ab to CD1b (10 μg/ml). The figure shows the results from one representative experiment of three.

CD1b and was equally prominent for the two additional isoforms of CD1, CD1a, and CD1c (data not shown).

To investigate the biologic implication of this finding, we tested the functional activity of CD1$^+$ APC infected with *M. tuberculosis*. We evaluated the capacity of infected cells to stimulate IFN-γ secretion from a CD1b-restricted T cell line at different time points after infection. DN.PT is a previously described CD4$^-$CD8$^-$ TCR $\alpha\beta^+$ cell line that specifically recognizes mycolic acids of *M. tuberculosis* and lyses CD1$^+$ APC infected with virulent *M. tuberculosis* (10). T cells were added to CD1$^+$ APC after 4 or 48 h of infection with live or dead *M. tuberculosis*. After 16 h of coincubation, the IFN-γ release was measured by ELISA. There was no difference in the IFN-γ secretion by DN.PT, whether mycobacterial Ag was presented by uninfected or 4-h infected CD1$^+$ APC (Fig. 4). However, after 48 h of infection with live mycobacteria, the IFN-γ secretion by DN.PT was completely diminished. In strong contrast, CD1$^+$ APC infected with heat-killed *M. tuberculosis* maintained their capacity to present Ag in a CD1b-restricted manner throughout the course of the infection. These studies indicate that infection with live, but not dead *M. tuberculosis* results in the functional down-regulation of CD1 from the cell surface of CD1$^+$ APC.

Down-regulation of CD1 is not due to a soluble factor

Infection of macrophages with *M. tuberculosis* induces the secretion of a variety of cytokines that have been implicated in reducing the capacity to mount an efficient immune response to mycobacteria. IL-10 has been shown to reduce the secretion of IFN-γ as well as the expression of the costimulatory molecule CTLA-4 on T cells (12, 13). In addition, IL-10 inhibits the up-regulation of CD1 on monocytes treated with GM-CSF and IL-4 (14). IL-6 is partly responsible for the inability of mycobacteria-infected cells to present exogenously added Ag to T cells (15). Finally, infection of monocytes with *M. tuberculosis* induces the secretion of TGF-β, which inhibits killing of intracellular mycobacteria (16).

Table I. *Down-regulation of CD1 is independent of cytokines*[a]

M. tuberculosis	Abs Added	Cytokines Added	CD1$^+$ Cells (%)
−	None	−	58
+	IL-10	None	16
+	TGF-β	None	13
+	IL-6	None	15
+	IL-12	None	10
+	TNF-α	None	13
+	None	None	12
−	None	IL-10	56
−	None	TGF-β	55
−	None	IL-6	59
−	None	IL-12	54
−	None	TNF-α	54

[a] CD1$^+$ cells were infected with live *M. tuberculosis* at an MOI of 10. After 4 h, extracellular bacteria were washed off and the incubation of the cells was continued in the presence of Abs against a panel of cytokines (all at 20 μg/ml). After 48 h, cells were harvested and CD1 expression on the cell surface was analyzed by FACS. Uninfected CD1$^+$ cells were, incubated in the presence of a panel of cytokines (all at 10 ng/ml). After 48 h cells were harvested and CD1 expression on the cell surface was analyzed by FACS. Data represent one experiment out of four with similar results.

We were interested in whether down-regulation of CD1 would be an additional feature of these immunosuppressive cytokines. CD1$^+$ APC were infected with *M. tuberculosis* and incubated in the presence of blocking Abs to these cytokines for 48 h. Abs to IL-10, TGF-β, or IL-6 were not able to inhibit the *M. tuberculosis*-induced down-regulation of CD1 (Table I). To exclude the possibility that we used an ineffective concentration, we titered the Abs in the range of 1 to 25 μg/ml and obtained similar results (data not shown). Growth of the intracellular mycobacteria was not influenced by the presence of the Abs (data not shown). In addition, Abs to IL-12 and TNF-α, both secreted by APCs after infection with *M. tuberculosis* (16–19), had no effect (Table I).

To confirm that cytokines induced by *M. tuberculosis* are not involved in the down-regulation of CD1, we added recombinant cytokines to uninfected CD1$^+$ APC for 48 h and asked whether they would affect expression of CD1. None of the cytokines tested (IL-10, TGF-β, IL-6, IL-12, TNF-α) had an effect on CD1 expression (Table I). Table I presents results using cytokine concentrations of 10 ng/ml; however, when concentrations were varied between 1 and 25 ng/ml, the results were similar (data not shown).

The possibility remained that additional cytokines or soluble factors secreted by infected CD1$^+$ APC or *M. tuberculosis* itself could contribute to the down-regulation of CD1. We incubated uninfected CD1$^+$ APC in the lower chamber of a transwell system separated from infected CD1$^+$ APC in the upper chamber by a size-restricting membrane (0.4 μm). Thereby, soluble mycobacterial and macrophage-derived components are able to traffic liberally between the chambers, while whole *M. tuberculosis* is excluded. No down-regulation was observed in the cells incubated in

Table II. *Down-regulation of CD1 is independent of secreted factors*[a]

Upper Chamber	Lower Chamber	CD1$^+$ Cells (lower chamber)
Infected CD1$^+$ cells	Uninfected CD1$^+$ cells	55
Uninfected CD1$^+$ cells	Infected CD1$^+$ cells	17
Uninfected CD1$^+$ cells	Uninfected CD1$^+$ cells	57

[a] Infected cells (1 × 10^6) were plated in the upper chamber of a transwell system. In the lower chamber, 3 × 10^6 uninfected CD1$^+$ APC were cultured. After 48 h, the cells in the lower chamber were detached with EDTA, and FACS staining for CD1b was performed. One representative experiment of six is shown.

Uninfected cells
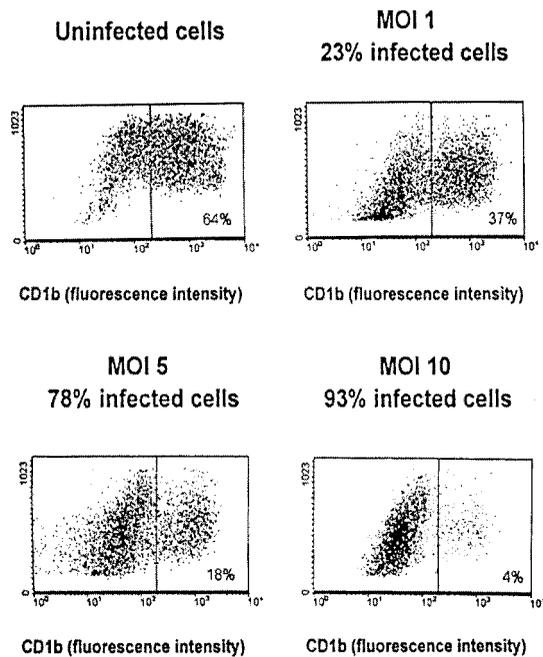
FIGURE 5. Effect of different MOIs on the expression of CD1b on CD1$^+$ APC infected with live *M. tuberculosis*. CD1$^+$ APC were infected with live *M. tuberculosis* using MOIs, as indicated. After 48 h, cells were detached and expression of CD1b was analyzed by flow cytometry with anti-CD1b FITC. The percentage of cells infected was determined by microscopic evaluation of Ziehl-Neelsen-stained samples. The figure shows the results from one representative experiment of three.

FIGURE 6. Expression of CD1 isoform mRNA in infected cells. CD1$^+$ cells were infected with live *M. tuberculosis* at an MOI of 10. After 48 h, cells were detached, and the amount of mRNA for CD1a, b, and c was compared between infected and uninfected cells. The β-actin cDNA product was used for standardization of input cDNA prior to amplification. The template cDNAs were serially diluted prior to PCR in order to eliminate the possibility of PCR amplification artifacts. The figure shows the results from one representative experiment of three.

the lower chamber of the transwell system, corroborating that soluble factors are not involved (Table II).
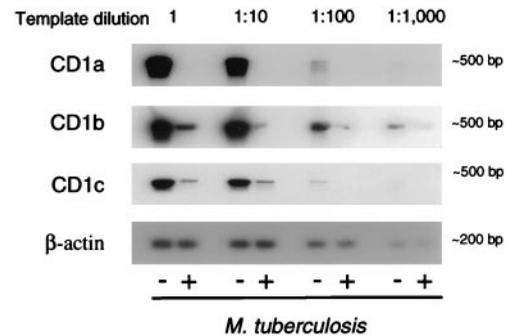
Since down-regulation of CD1 occurs independently of secreted factors, we hypothesized that the immediate interaction of *M. tuberculosis* with its host cell is a precondition for the down-modulation of CD1. To test this, we infected CD1$^+$ APC at different MOIs to achieve graduated amounts of infected cells. The number of infected cells correlated very well with the extent of down-regulation of CD1 (Fig. 5). At an MOI of one, 23% of the cells were infected and the number of CD1$^+$ cells decreased from 64 to 37%. With increasing numbers of bacteria resulting in a higher efficiency of infection, the surface CD1 expression declined gradually until finally dropping to background levels when 93% of the cells were infected. These data confirm our earlier findings and suggest that down-regulation of CD1 requires the active interaction of *M. tuberculosis* with the host cell machinery.

Mechanism of the down-regulation of CD1

The lack of expression of CD1 on the cell surface could be a consequence of the inhibition of RNA transcription. To investigate this possibility, we synthesized cDNA from cultures of CD1$^+$ APC infected with live *M. tuberculosis* for 48 h, and performed reverse-transcription PCR to compare the mRNA expression of CD1a, b, and c between infected cells and uninfected controls. The striking finding was that infection with *M. tuberculosis* induced an almost complete disappearance of mRNA for the three CD1 isoforms (Fig. 6).

Discussion

Understanding the evasion strategies that allow intracellular pathogens to persist within the cell requires knowledge of the mechanisms resulting in the inability of the host immune system to detect

infected cells. The present study was designed to investigate whether intracellular pathogens interfere with the expression and function of the recently characterized nonclassical Ag-presenting molecule CD1. Using *M. tuberculosis* as a prototypic pathogen with the capability to persist within macrophages, we describe and characterize the down-regulation of CD1 on CD1$^+$ APC by live mycobacteria. This resulted in the inability of protective CD1-restricted T cells to recognize mycobacterial lipid Ags on the cell surface of the host monocytes. These data introduce a new mechanism utilized by an intracellular human pathogen to evade the immune response.

Our study is novel in that it characterizes the impact of an infection with *M. tuberculosis* on CD1$^+$ APC. We infected the adherent fraction of PBMC, which had been treated with GM-CSF and IL-4, with virulent *M. tuberculosis*. These cells have morphologic features and a cell surface phenotype (Fig. 1) that closely resemble immature dendritic cells (7, 8). There are several lines of evidence that cells belonging to the dendritic cell lineage are main players in the immune response to infection with intracellular pathogens. First, dendritic cells previously have been shown to phagocytose not only soluble mycobacterial Ag (20), but also whole parasites (21) and bacteria (22, 23), including Bacille Calmette Guerin (24), and *M. tuberculosis* (25). Second, immunohistologic studies demonstrated an accumulation of CD1$^+$ cells in the granulomas of patients with the immune-responsive, tuberculoid form of leprosy (P. Sieling et al., submitted for publication) as well as in biopsies of patients with tuberculous lymphadenitis (S. Stenger and P. Barnes, unpublished observation). Third, in vivo studies have implicated cells of the dendritic cell lineage in the lymph nodes of infected mice as a safe target for persisting *Leishmania major* (26, 27). However, the mechanism of parasite persistence in the APC of chronically infected hosts remained unresolved. Our studies provide first evidence for an evasion strategy that could account for the ability of a live organism to persist within CD1$^+$ cells. In contrast, in dendritic cells cultured for 7 days, CD1 expression is relatively low and is not reduced by infection with *M. tuberculosis*, as reported by Henderson et al. (25).

These data could be interpreted to suggest that in a subject infected with *M. tuberculosis*, down-regulation of CD1 expression would prevent CD1-Ag presentation. However, *M. tuberculosis*-reactive CD1-restricted T cells have been identified in patients with tuberculosis (10, 28). These CD1-restricted T cells have been shown to lyse APC infected with virulent *M. tuberculosis* rapidly

within 4 h after the initial contact (10). The process of lysing the infected target cell can directly or indirectly result in the death of the bacteria (10). We hypothesize that the majority of cells infected with *M. tuberculosis* will be eliminated during the initial attack of the immune system initiated by the combined action of $\gamma\delta$ T cells (29, 30), as well as MHC class I/II (31, 32) and CD1-restricted T cells. The down-regulation of CD1, as described in this work, will only occur in those cells, which have managed to escape this initial immune response. It is therefore conceivable that CD1-restricted Ag presentation contributes to the protective immune response to *M. tuberculosis* during the acute stage of infection, whereas the few persisting bacteria will lead to the down-regulation of CD1 during the chronic stage, converting CD1$^+$ APCs into safe compartments for the pathogen.

Our data show that down-regulation of CD1 on CD1$^+$ APC only occurs if the cells are infected with live *M. tuberculosis*. In contrast, heat-killed mycobacteria did not affect CD1 expression, indicating that down-regulation of CD1 might require specific interactions between live *M. tuberculosis* and the host cell machinery. Indeed, the intracellular trafficking pathway between virulent and heat-killed *M. tuberculosis* involves distinct compartments (33). In addition, only live *M. tuberculosis* have been suggested to form a pore into their endosomal compartment, thereby facilitating MHC class I-restricted presentation of soluble Ags (34). By this mechanism, components of multiplying mycobacteria could escape from the endosome into the cytosol and affect signal-transduction pathways responsible for CD1 expression.

Our study demonstrates that the down-regulation of CD1 is unlikely to be an effect of a soluble mediator. First, the down-regulation of CD1$^+$ APC cannot be achieved by addition of recombinant cytokines nor be reversed by incubation with Abs that block cytokine function. Second, the down-regulation is not observed in a transmembrane system, where infected CD1$^+$ APC are separated from noninfected cells by a membrane that is only permeable for soluble mediators, but not bacteria. Third, the number of infected cells correlates with the number of cells that down-regulate the CD1 molecule, as demonstrated by the bimodal distribution of the fluorescence for CD1b (Fig. 5). Our data suggest that the down-regulation of CD1 is not the consequence of nonspecific inhibition of surface molecule expression by infection with live *M. tuberculosis*. Importantly, the levels of MHC class I and class II did not change significantly in response to infection (Fig. 4), confirming previous studies using mycobacteria-infected macrophages (15, 35, 36).

Our data indicate that the down-regulation of surface CD1 expression occurs at the mRNA level, either by the regulation of transcription or increased degradation. Since CD1b expression on stably transfected THP-1 cells (a human monocytic cell line) using a heterologous promoter was not affected by *M. tuberculosis* infection (data not shown), we propose that the differential regulation of CD1 is occurring during gene transcription and is independent of mRNA stability or processing. *M. tuberculosis* infection of monocytes has been suggested to affect gene transcription via inhibition of the nuclear translocation of the transcription factor STAT1 in other studies (37). Another example of down-regulation of the expression of mRNA encoding an Ag-presenting molecule by infection with a pathogenic organism has been demonstrated previously, specifically, dendritic cells infected with Rauscher Leukemia virus exhibit decreased MHC class II mRNA levels (38). Analysis of CD1 transcription and mRNA stability should provide insight into the mechanism by which *M. tuberculosis* decreases CD1 mRNA.

Several evasion strategies of *M. tuberculosis* have been characterized. In particular, mycobacteria inhibit lysosomal acidification by inhibiting the proton ATPase (39, 40), prevent the fusion of

phagosomes with lysosomes (33, 41, 42), circumvent immune recognition by CD4$^+$ cells by sequestering Ag (36), and impair accessory functions of infected monocytes in part by down-regulating HLA-DR (43). In addition to these direct interactions of mycobacteria with intracellular organelles, they also deactivate defense mechanisms of macrophages indirectly by inducing the secretion of immunosuppressive cytokines such as IL-10, TGF-β, and IL-6 (15, 16). Our study demonstrates that live *M. tuberculosis* inhibits the functional expression of the Ag-presenting molecule CD1. The characterization of this novel interaction between *M. tuberculosis* and the host cell extends our understanding of the ability of intracellular pathogens to persist in the host.

Acknowledgments

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