Interaction of *Mycobacterium avium* with Human Monocyte-Derived Dendritic Cells

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The mechanism by which mycobacteria elicit class I-restricted T-cell responses remains undefined because these organisms have been shown to reside exclusively within membrane-bound vesicles in macrophages (Mφ), their primary host cells. We studied the interaction of *M. avium* with dendritic cells (DC) because they are the most potent antigen-presenting cells and are abundant at *M. avium* infection sites. We observed that both DC and Mφ, generated from human peripheral blood monocytes by short-term culture, internalized *M. avium*. The onset of programmed cell death and the percentage of apoptotic cells in infected DC and Mφ were comparable. However, following infection, DC secreted significantly larger amounts of interleukin-12, but not interleukin-1β, than infected autologous Mφ. Further analysis of infected cells showed that while phagosomes failed to acidify in both *M. avium*-infected DC and Mφ, bacilli grew more slowly in DC. Electron microscopy studies revealed that *M. avium* resided within endocytic vacuoles in both cell types. The vacuolar membrane surrounding some bacilli in approximately 10% of the vacuoles in DC possessed several breaks. The importance of this finding will have to be addressed in future studies.

Organisms of the *Mycobacterium avium* complex are rarely pathogenic for healthy individuals (17) but cause disseminated disease in patients with AIDS (24, 40) and localized pulmonary infection in non-AIDS patients with underlying chronic lung disease (26). Infection with *M. avium* poses staggering public health problems because of the limited susceptibility of this organism to available antibiotics (12) and the ability of these bacilli to become resistant to commonly used antituberculosis agents (19). To develop new strategies for treating *M. avium* infection, we need to better understand the interactions of this organism with the host's immune system.

As is the case with other mycobacteria, the importance of CD8+ T cells in resistance to *M. avium* is controversial (6, 27) because the mechanism by which *M. avium*-derived molecules gain access to the cytoplasmic presentation pathway to elicit major histocompatibility complex class I-restricted T cells remains undefined. It is widely accepted that *M. avium*, a facultative intracellular bacillus, impedes macrophages' (Mφ) processing and presentation of antigen by restricting vacuole maturation (10, 36, 39). In view of the paradox concerning the involvement of mycobacterium-specific major histocompatibility complex class I-restricted T cells in the control of infection and the finding that *M. avium* remains primarily within membrane-bound vesicles in Mφ, we analyzed human dendritic cells (DC) infected in vitro with *M. avium* for uptake and intracellular growth of bacilli, apoptotic death, production of interleukin-12 (IL-12), fusigenicity of bacilli containing vacuoles with lysosomes, and the intracellular localization of the bacteria. The belief that *M. avium* may display behavior in DC different from its behavior in Mφ was inspired by studies show-ing that in DC there is a rapid fusion between the *Chlamydia* vacuoles and host cell lysosomes (25). *Chlamydia* is known to survive within its primary host cells, epithelial cells, through its ability to inhibit fusion between the entry vacuoles and host cell lysosomes (31).

DC are potent antigen-presenting cells (APC) (35) and show clear superiority particularly in inducing primary immune responses to other APC types, including Mφ (23, 34). DC are present in the airway epithelium, lung parenchyma, and visceral pleura (33) and may contribute to generating *M. avium*-specific CD4+ and CD8+ T-cell responses. They are among the first cells to encounter a pathogen and have the capacity to internalize it and process its antigens before migration to secondary lymphoid organs (1). Cells of dendritic lineage are also a major source of IL-12 (32). IL-12 favors the development of CD4+ T-helper 1 cells (38). In mice, endogenous IL-12 is required for resistance to *M. avium* infection (28), and administering recombinant IL-12 augmented resistance to *M. avium* infection in susceptible mice (11, 20). In keeping with these findings, the adherent cells from patients with familial disseminated *M. avium* complex infection were shown to have a defect in IL-12 production (14).

Our results show that DC, generated from human peripheral blood mononuclear cells (PBMC) by short-term culture, internalize *M. avium*. The percentages of apoptotic cells in *M. avium*-infected DC and Mφ were comparable. Following infection with *M. avium*, DC secreted higher amounts of IL-12, but not of IL-1β, than autologous Mφ. Like Mφ, the DC phagosomes containing *M. avium* did not acidify; however, the organisms grew more slowly in DC. Electron microscopy studies revealed that *M. avium* resided within endocytic vacuoles in both cell types.

MATERIALS AND METHODS

Antibodies. Monoclonal antibodies (MAb) to CD4 (Leu-3a), CD14 (Leu-M3), CD19 (Leu-12), and HLA-DR (CA141) were generously provided by Edgar G. Engleman (Stanford University School of Medicine, Stanford, Calif.). We pur-

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chased from Becton-Dickinson Monoclonal Center (Mountain View, Calif.) and PharMingen (San Diego, Calif.) phycoerythrin (PE)-conjugated MAb directed at CD3, CD4, CD14, CD16, CD20, CD33, CD80, CD86, and HLA-DR, as well as PE-labeled isotype-matched immunoglobulin G. Affinity-purified goat anti-mouse immunoglobulin G (γ-chain-specific) antibody was purchased from Caltag Laboratories (Burlingame, Calif.).

Bacteria. *M. avium* 101 (serotype 1) was originally obtained from the blood of an AIDS patient. For the experiments, *M. avium* was cultured on Middlebrook 7H10 agar and transparent colonies were expanded by an additional 5 days' incubation in 7H9 broth supplemented with oleic acid, dextrose, albumin, and catalase.

Mycobacterium smegmatis mc²155 was cultured in the manner described above; however, it was harvested after 3 days in culture. *Escherichia coli* HB101 was cultured on Mueller-Hinton agar for 24 h and subsequently for 4 h in Mueller-Hinton broth under constant agitation.

For the experiments, mycobacteria were washed in Hanks balanced salt solution and the final suspension was passed through a 23-gauge needle 10 times and then vortex agitated for 1 min. The suspension in a 15-ml polystyrene tube was allowed to rest for 5 min at room temperature, and the top 5 ml (dispersed inoculum) was obtained and adjusted to 10^8 bacilli using a McFarland standard (4). Bacterial viability was determined using the LIVE-DEAD assay (Molecular Probes, Eugene, Oreg.) as described (4).

Culture medium. DC were cultured in AIM-V medium (Life Technologies, Inc., Grand Island, N.Y.) that was supplemented with 5% human AB serum, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 0.1 mM nonessential amino acid, 100 μg of streptomycin/ml, 100 U of penicillin/ml, 2.5 μg of amphotericin B (Fungizone)/ml, 2,000 U of recombinant human granulocyte-Mφ colony-stimulating factor (rhGM-CSF) (Immunex, Seattle, Wash.)/ml, and 30 ng of rhIL-4 (Caltag Laboratories)/ml (hereafter designated DC complete medium [DC-CM]).

Mφ were cultured in Macrophage-SFM (Life Technologies, Inc.) supplemented with 5% human AB serum, 100 μg of streptomycin/ml, 100 U of penicillin/ml, 2.5 μg of amphotericin B/ml, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acid (hereafter designated Mφ complete medium [(Mφ-CM]).

T cells were cultured in AIM-V medium supplemented only with 5% human AB serum, 100 μg of streptomycin/ml, 100 U of penicillin/ml, 2.5 μg of amphotericin B/ml, and 2 mM L-glutamine.

Isolation of Mφ and T cells. PBMC were isolated by Histopaque-1077 (Sigma) gradient centrifugation from the white-cell concentrate purchased from the Blood Center of the Pacific (San Francisco, Calif.). Monocytes were isolated from PBMC by their differential densities (21). Briefly, PBMC were separated into low-density and high-density Percoll (Pharmacia Biotech, Uppsala, Sweden) fractions. Low-density cells (monocytes), collected from the interface over 51% Percoll solution, were refloated on a second Percoll gradient for further enrichment.

CD4+ T cells were obtained from the T-cell-enriched, high-density Percoll fraction of the same blood drawing used for Mφ isolation. This fraction was depleted of B cells, activated (HLA-DR+) T cells, DC, and Mφ by panning (23) using a mixture of anti-CD19, anti-HLA-DR, and anti-CD14 MAb. Residual Mφ were removed from the nonadherent fraction by overnight adherence in plastic vessels at 37°C. A final positive-selection panning procedure utilizing anti-CD4 MAb yielded cells that were ≥97% CD4+.

Cell culture. First, 25×10^6 to 30×10^6 purified monocytes were cultured in T75 flasks (Costar, Cambridge, Mass.) in 30 ml of either DC-CM or Mφ-CM. After overnight incubation at 37°C in a humidified atmosphere containing 5% CO_2, the medium of each culture was changed to remove the residual nonadherent cells. Thereafter, the medium was replaced every 4 days, and the nonadherent cells, recovered by centrifugation of the old medium, were added back to the cell culture. After 8 and 12 days, the nonadherent cells were harvested for analysis.

Fluorescence-activated cell sorter analysis. Approximately 2.5×10^5 cells were stained with the indicated PE-conjugated MAb by standard techniques (23). After thorough washing, each sample was fixed with 1% paraformaldehyde and analyzed within 3 days on a FACS Calibur (Becton-Dickinson, San Jose, Calif.). The staining intensity of a particular MAb was evaluated relative to an isotype-matched control MAb by analyzing 5,000 cells. Data were measured in log scale.

Proliferation assay. All proliferation assays were performed in round-bottomed microtiter wells in a final volume of 200 μl of T-cell medium. In these experiments, 50×10^3 cryopreserved autologous (for induction of an autologous mixed lymphocyte reaction) or 50×10^3 allogeneic (for induction of an allogeneic mixed lymphocyte reaction [MLR]) CD4+ T cells were incubated with varying numbers of irradiated (3,000 rads from a ^{137}Cs source) DC or Mφ. Control T cells were incubated in medium alone. All cultures were carried for 6 days at 37°C in a humidified 10% CO_2 atmosphere. Cellular proliferation was measured on the basis of uptake of [^{3}H]thymidine that was added 16 h before harvesting. Results are the mean counts per minute ± standard errors of the means (SEM) of four replicate cultures.

Infection of Mφ and DC. DC (5×10^5) were incubated in a Lab-Tek chamber (Nunc, Inc., Naperville, Ill.) in antibiotic-free DC-CM (rhGM-CSF only) containing 10% nonheated human AB serum. These cells were infected with 5×10^6 (multiplicity of infection [MOI] of 10) bacteria for 1 or 4 h. After the infection period, the culture supernatant was removed and monolayers were washed care-

fully three times with 37°C warmed Hanks balanced salt solution to ensure removal of the extracellular bacteria. Mφ were incubated in antibiotic-free Mφ-CM as described for DC. To determine the *M. avium* uptake, cells were lysed with sterile water and 0.25% sodium dodecyl sulfate. The lysate was diluted and plated onto 7H10 agar as reported (4). In some assays, extracellular bacteria were removed by washing and intracellular bacteria were allowed to replicate for several days (4). At different times, the assay was stopped by lysing the monolayers, and serial dilutions of the cell lysate were plated onto 7H10 agar for quantitation of intracellular bacteria (4).

Cytokine assay. Mφ and DC monolayers were infected with *M. avium* for 24, 48, and 72 h before supernatants were collected, filtered through a 0.22-μm-pore-size filter, and frozen. Some control wells were treated with polymyxin B (10 μg/ml) to ensure that mycobacteria, but not the contaminating lipopolysaccharide (LPS), stimulated cytokine production. Concentrations of IL-12 (p70) and IL-1β in the supernatant of cell culture were measured by enzyme-linked immunosorbent assay (Biosource International, Camarillo, Calif.). The lowest limit of detection was 5 pg/ml for IL-1β and 10 pg/ml for IL-12. The assays were repeated twice, and three samples were collected in each assay.

pH measurement. *M. avium* labeled with *N*-hydroxysuccinimide (NHS)–carboxyfluorescein (Sigma Chemical Co.) was used to infect DC and Mφ as previously described (36). Extracellular bacteria were removed by washing. The fluorescence of the total cell population was measured at different times fluorometrically and compared with a standard pH curve constructed using *N*-hydroxysuccinimide–carboxyfluorescein-labeled *M. avium* in suspension and in nigericin-treated Mφ and DC (36).

A second method required lysosensor pH indicators (Molecular Probes). Lysosensor Blue DND-167 is almost nonfluorescent except in acidic conditions, whereas Lysosensor Blue DND-192 is brightly fluorescent at neutral pH. Mφ and DC were infected, and the pH probes were added at different times. Monolayers were washed after 30 min, and the fluorescence emission was determined. DND-167 was read at A_{373} and an emission of 425 nm, while DND-192 was read at A_{354} and an emission of 454 nm. A standard curve was constructed in parallel using different pH references.

Phagosome-lysosome fusion. We used the following two assays to determine if *M. avium* remains within a vacuole following uptake by DC.

(i) DC and Mφ after 8 or 12 days of in vitro differentiation were transferred into a Lab-Tek chamber before infection with either *M. avium*, *M. smegmatis*, or *E. coli*. Two hours after infection, extracellular bacteria were removed by washing and the medium was replaced. Eighteen hours after infection, cells were incubated with acridine orange (Sigma Chemical Co.) for 15 min as previously reported (2, 7). After the incubation, monolayers were washed to remove the excess of acridine orange and then the slides were rapidly mounted and observed under a light microscope for 30 min. After 30 min, the intralysosomal acridine orange began to diffuse to the rest of the cell (2, 7). The number of bacteria that were stained by the acridine orange (fused vacuoles) was counted in 200 cells.

(ii) *M. avium* was used to infect both Mφ and DC monolayers on coverslips. At several time points, the monolayers were fixed with 2% glutaraldehyde and processed for electron microscopy as previously described (4, 7).

Apoptosis assay. To determine whether DC infected with *M. avium* undergo apoptosis, monolayers of both DC and Mφ were infected with *M. avium*, and the number of apoptotic cells in each culture was determined by both ELISA and terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay at days 3, 5, and 7 postinfection as previously described (4, 5).

Electron microscopy. *M. avium*-infected DC and Mφ were fixed with ice-cold 1% glutaraldehyde in phosphate buffer for 1 h and postfixed in 1% aqueous osmium tetroxide for 1 h at 4°C as described (4). They were dehydrated in ethanol at room temperature, embedded in resin, and polymerized at 52°C. Ultrathin sections were cut and mounted on carbon-coated grids. The distribution of bacteria per vacuole was scored by examining 2 to 10 vacuoles for each cell type.

Statistical analysis. The results of the experiments are expressed as means ± SEM. Significance between experimental groups and controls was analyzed by the Student *t* test. A *P* value of <0.05 was considered significant.

RESULTS

Characterization of DC and Mφ. Using a multistep procedure described by Markowicz and Engleman (21), we obtained highly enriched monocytes from human peripheral blood. Cytofluorographic analysis of freshly isolated monocytes (low-density Percoll fraction) indicated that ≥94% were stained with anti-CD14 MAb, ≥95% were stained with anti-HLA-DR MAb, ≥73% expressed CD33 molecules (a myeloid marker), and ≤4% were CD3+ or CD20+ (data not shown).

After incubation with GM-CSF and IL-4, human CD14+ monocytes differentiated into "immature" DC, as evidenced by their morphology, surface phenotype, and ability to induce autologous T cells to proliferate in an MLR (21). These cells tended to aggregate, displayed an array of cell processes, and

reversibly adhered to plastic. The phenotype of DC generated in this manner from six blood donors was different from that of Mφ (monocytes maintained in culture in the absence of exogenous cytokines for the same length of time). Unlike Mφ, a few (3 to 6%) cells in the DC population displayed CD14 molecules. A higher percentage of DC were CD80⁺ (33% ± 8% versus 3% ± 1%, $P < 0.05$). DC displayed significantly ($P < 0.01$) higher MFI for the CD86 molecule than did Mφ (138 ± 22 versus 31 ± 5). Despite the wide donor variation in the MFI value for HLA-DR molecules, the expression of these molecules by DC and Mφ was similar (70 ± 16 and 63 ± 11, respectively).

DC, but not Mφ, were able to induce proliferation in purified autologous CD4⁺ T cells (Fig. 1). DC were also more potent stimulators of allogeneic T-cell proliferation in the MLR ($P < 0.01$) than were Mφ. This difference was particularly pronounced at a low stimulator/responder ratio of 1:20 (Fig. 1). The ability of DC to stimulate T-cell proliferation in MLR depends partly on the increased expression of the costimulatory molecules CD80 and CD86 (30).

Fate of *M. avium* in DC and Mφ. To compare the abilities of monocyte-derived DC and Mφ to internalize *M. avium*, (1.5 ± 0.4) × 10⁶ organisms were incubated with (1.2 ± 0.3) × 10⁵ cells on monolayers for 1 and 4 h. Following incubation, monolayers were washed, cells were lysed, and liberated bacteria were enumerated by colony count. Mφ consistently ingested greater (but not statistically significant) numbers of bacteria than did DC after both 1 h of incubation [(1.6 ± 0.5) × 10⁵ versus (9.1 ± 0.4) × 10⁴ CFU] and 4 h of incubation [(4.9 ± 0.3) × 10⁵ versus (2.1 ± 0.4) × 10⁵ CFU] ($n = 5$). Because the extent of DC differentiation may influence their functional capacities, we infected DC from the same blood donor after 8 and 12 days of in vitro differentiation. The results showed that cells cultured for 12 days ingested numbers of bacteria similar to those ingested by cells cultured for 8 days (data not shown).

To compare the fate of *M. avium* in DC and Mφ, infected cells were recultured for 1 to 72 h in fresh antibiotic-free medium before the number of viable bacilli in each cell type was determined by colony count. *M. avium* was able to replicate within both monocyte-derived DC and Mφ; however, DC markedly restricted replication of the bacterium compared to Mφ (4-fold increase in DC versus 10-fold increase in Mφ) (Table 1).

Phagosome-lysosome interaction in DC and Mφ. To determine if *M. avium* prevents phagosome-lysosome fusion when taken up by DC, we infected DC with *M. avium* for 18 h before staining the monolayers with acridine orange. Mφ infected with *M. avium* were used as control because restricted fusigenicity of *M. avium* vacuoles with lysosomes in these cells has been reported (36). The numbers of fused vacuoles were comparable in DC and Mφ, as shown in Fig. 2.

Control DC and Mφ infected for 18 h with *M. smegmatis* or for 4 h with *E. coli* showed significant fusion of phagosomes and lysosomes.

***M. avium*-containing phagosomes in DC are unable to acidify.** *M. avium* within Mφ has been observed to live in vacuoles that do not acidify (36, 39). In this study, we compared the *M. avium* environments in DC and Mφ by measuring the intraphagosomal pH in both cell types at 4, 24, and 48 h after infection. In Mφ, the pH in *M. avium* vacuoles did not drop below 6.7 at any time. The vacuoles in DC infected with *M. avium* either 8 or 12 days after in vitro differentiation also failed to acidify (pH of ≥6.7 ± 0.3 versus 6.9 ± 0.2 in the uninfected DC and Mφ). Within 4 h of *M. smegmatis* internalization, the vacuoles in both DC and Mφ had pH values of 5.6 ± 0.1 and 5.4 ± 0.1,

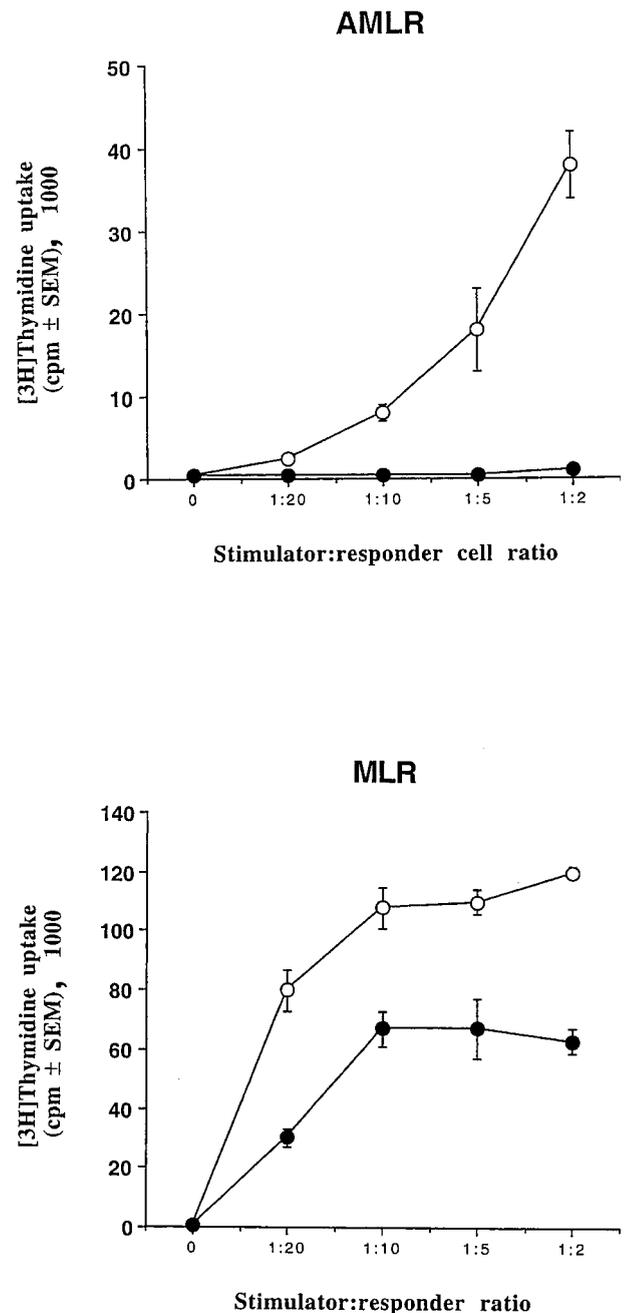
FIG. 1. DC are potent T-cell stimulators. Varying numbers of irradiated DC (open circles) or Mφ (solid circles) were used to stimulate 5 × 10⁴ autologous or allogeneic CD4⁺ T cells as described in Materials and Methods. [³H]thymidine uptake by T cells incubated without the stimulator cells was ≤300 cpm. AMLR, autologous mixed lymphocyte reaction.

respectively ($P < 0.05$ compared to that of *M. avium*-infected cells).

IL-12 production by DC. To determine if Mφ and DC differ in their ability to produce IL-12 following *M. avium* internalization, DC and Mφ that were allowed to differentiate in vitro for 8 days were infected with *M. avium* and the level of IL-12 in the culture supernatant was measured after 24, 48, and 72 h. Production of IL-1β was assessed as well. We conducted these experiments because IL-12 production is the centerpiece of the specific immune response, and APC such as DC and Mφ produce IL-12 upon antigen uptake.

TABLE 1. Replication of *M. avium* within DC and Mφ[a]

Time point (h)	No. of intracellular bacteria (10^5) (mean ± SEM) in	
	DC[b]	Mφ
1	2.1 ± 0.4	3.6 ± 0.3
24	3.8 ± 0.3	7.1 ± 0.4
48	7.7 ± 0.5	13 ± 0.4
72	9.3 ± 0.4	37 ± 0.2

[a] Monocyte-derived DC and Mφ from five healthy blood donors were infected with *M. avium* at an approximate MOI of 10. The inoculum contained (2.1 ± 0.3) × 10^6 CFU. After 1 h of incubation, cells were washed and recultured in fresh medium as described in Materials and Methods. At the indicated time points, the number of viable bacilli in the cell lysates was determined by colony counts.
[b] $P < 0.05$ compared with the number of bacteria in Mφ at the same time point.

DC infected with *M. avium* produced significantly greater amounts of IL-12, but not of IL-1β, than did Mφ (Table 2). We carried out a similar experimental protocol using medium that contained 10 μg of polymyxin B/ml because contamination with LPS influences IL-12 production. The results showed that LPS contamination was not responsible for IL-12 production.

DC apoptosis. Data from a number of studies have indicated that Mφ prevent the spread of infection by undergoing apoptosis following mycobacterial infection (6, 13). We carried out comparative experiments with DC and Mφ to determine the degree of apoptosis occurring in these cells over time. As shown in Fig. 3, the percentage of cells undergoing apoptosis did not differ for DC and Mφ. Twenty percent of DC were apoptotic after 5 days of infection, compared to 4% of cells in the noninfected cultures, and the percentage of apoptotic DC increased with time.

Electron microscopy. The information available thus far indicates that both *M. avium* and *Mycobacterium tuberculosis* live within vacuoles in Mφ (36). We used transmission electron microscopy to examine if *M. avium* behavior in DC was similar to that previously described in Mφ. Electron micrographs of DC infected for 24 h with *M. avium* show that mycobacterium-
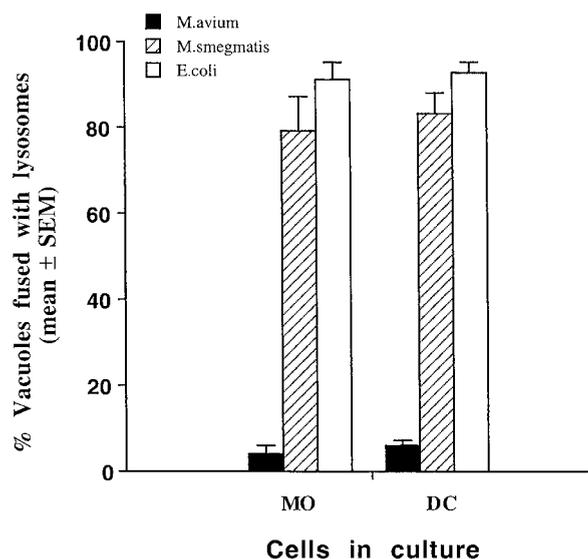
TABLE 2. IL-12 and IL-1β production in *M. avium*-infected DC and Mφ[a]

Treatment regimen	Time (h)	Mean concn ± SEM (pg/ml) in:			
		DC of:		Mφ of:	
		IL-12[b]	IL-1β	IL-12	IL-1β
M. avium	24	4,389 ± 210	176 ± 22	266 ± 16	197 ± 39
	48	1,136 ± 58	194 ± 41	214 ± 31	181 ± 22
	72	1,202 ± 83	136 ± 16	94 ± 3	148 ± 27
M. avium + polymyxin B	24	4,031 ± 163	186 ± 31	284 ± 34	187 ± 41
	48	2,264 ± 76	172 ± 23	273 ± 12	176 ± 32
	72	1,155 ± 60	131 ± 17	81 ± 18	150 ± 12

[a] As described in Materials and Methods, 5 × 10^5 DC or Mφ were infected with *M. avium* in the absence or presence of 10 μg of polymyxin B/ml at an MOI of 10. Concentrations of IL-12 and IL-1β in the culture supernatants collected at the indicated times were measured by enzyme-linked immunosorbent assay. Results are the means ± SEM of two independent experiments. The number of cells in the monolayer did not differ significantly in each experiment. The concentrations of IL-12 and IL-1β in the culture supernatants from noninfected cells were below the sensitivity of the assay (10 pg/ml for IL-12 and 5 pg/ml for IL-1β).
[b] $P < 0.05$ compared with the amount of IL-12 produced by Mφ at the same time point.

containing vacuoles did not fuse with lysosomes. *M. avium* phagocytosed by DC remained in tightly opposed vacuoles; however, in very few vacuoles of a small percentage of DC, we observed some sort of fracture of the vacuole membrane. The meaning of this finding is unknown and will have to be addressed in the future.

DISCUSSION

In this study, we analyzed the interaction between *M. avium* and DC generated by short-term culture from human peripheral blood monocytes, and we compared the results with the key features of internalization of *M. avium* by Mφ. We show that the ability of DC to take up *M. avium* and undergo apoptosis is approximately the same as that displayed by Mφ. Following internalization of bacilli, DC secreted higher

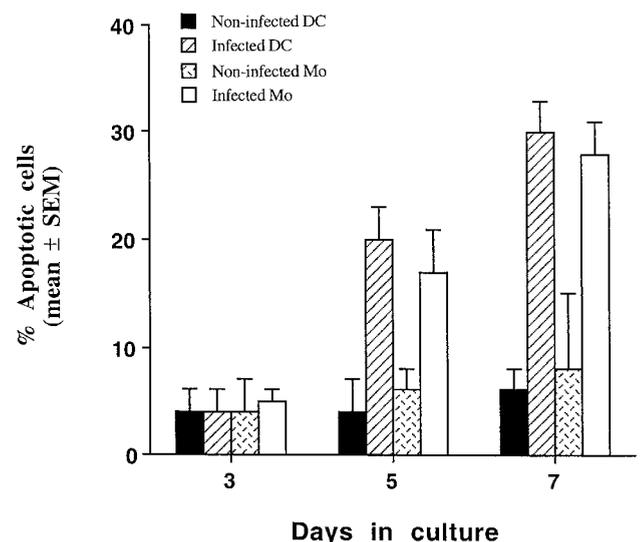FIG. 2. Phagosome-lysosome fusion in DC and Mφ. DC and Mφ were infected with either *M. avium*, *M. smegmatis*, or *E. coli* HB101 as described in Materials and Methods. The results are given as the percentage of vacuoles fused with lysosomes, counting 200 cells. Mo, Mφ.

FIG. 3. *M. avium* induces apoptosis in DC. Both DC and Mφ were infected with *M. avium* at an approximate MOI of 10. Noninfected DC and Mφ served as controls. The percentage of apoptotic cells in each culture was determined on indicated days by terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay. The data represent means ± SEM of three experiments.
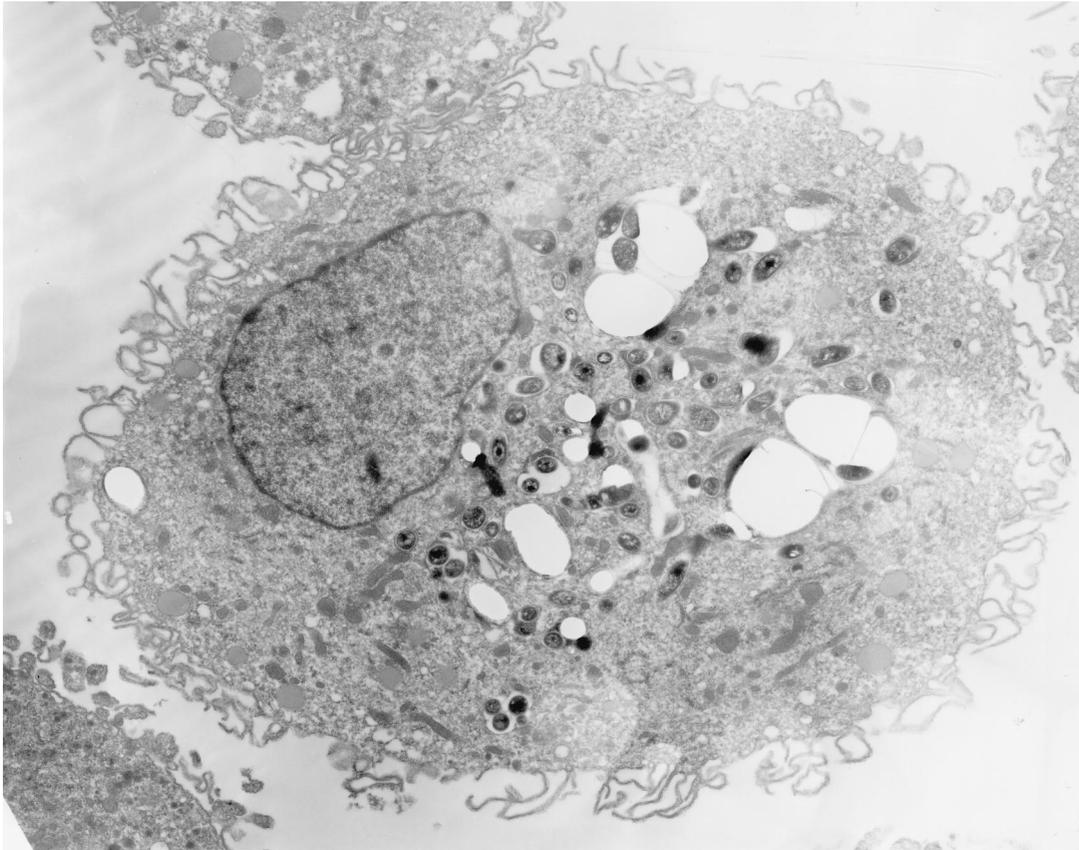
FIG. 4. Electron micrograph of human monocyte-derived DC infected with *M. avium*. Cells were processed 24 h after infection. The bacteria (large numbers of them) are seen inside tightly opposed, as well as large, vacuoles (magnification of ×6,187.5).

amounts of IL-12, but not of IL-1β, than autologous Mφ (Table 2). Like Mφ, the DC phagosome containing *M. avium* did not acidify; however, the organisms grew more slowly in DC. Although *M. avium* resided in tight cytoplasmic vacuoles in both DC and Mφ, the vacuolar membrane surrounding the bacilli in DC possessed several breaks which were not observed in Mφ (Fig. 4).

Our findings in DC are in agreement with the earlier observations by Henderson et al. (16), who showed that human monocyte-derived DC can phagocytose *M. tuberculosis* and that infection with this organism results in secretion of IL-1β and IL-12. The reason for the secretion of higher amounts of IL-12 by *M. avium*-infected DC than by autologous Mφ is unknown; however, data suggest a significant role for DC in the host defense against *M. avium*. Both in humans and in mice, IL-12 is required for resistance to *M. avium* (8, 11, 14, 20, 28). IL-12 augments cell-mediated immunity by stimulating production of gamma interferon and proliferation of T lymphocytes as well as of NK cells (9, 15). Infection of Mφ with *M. avium* does not result in a strong IL-12 response (D. Wagner, F. J. Sangari, M. Petrofsky, L. S. Young, and L. E. Bermudez, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1596, 1999).

Infection of DC with *M. avium* resulted in a significant increase in apoptotic death from 5 days after infection (Fig. 3), which is comparable with the onset of apoptosis in *M. avium*-infected Mφ (4, 5). Fratazzi et al. (13) have suggested that apoptosis of infected cells may represent a mechanism of host defense, although the interpretation of this observation remains controversial.

The reason for restricted growth of *M. avium* in DC (Table 1) remains to be determined. It is plausible that the intravacuolar environment in DC provided less favorable growth conditions than those provided in Mφ. Alternatively, in this study the slow bacterial replication in DC may have resulted from the exposure of these cells to low concentrations of rhGM-CSF during the initial in vitro incubation. A previous study from our laboratory demonstrated that GM-CSF enhances the anti-*M. avium* activity of human monocyte-derived Mφ in a dose-dependent manner (3). Schaible et al. (29) have also observed that cytokine activation of Mφ leads to fusion of the bacillus-containing compartments with lysosomes, culminating in the death of intracellular bacteria.

Electron microscopy studies, showing that in DC *M. avium* is found inside vacuoles, correspond with the recent finding for *M. tuberculosis* (16). Our observation that some (approximately 10%) vacuoles had breaks in a small percentage of cells is intriguing and will require further investigation. It has been suggested that mycobacterial antigens undergo exchange with the cytoplasm. This assertion is supported by (i) immunoelectron microscopy studies of *M. avium*- and *M. tuberculosis*-infected Mφ by Xu et al. (39), which demonstrated the presence of vesicles containing mycobacterial constituents (discrete from the bacterium-containing vacuoles), and (ii) the observation by Mazzaccaro et al. (22) and Teitelbaum et al. (37) that *M. tuberculosis* and *Mycobacterium bovis* BCG facilitate bidirectional exchange of macromolecules between cytoplasmic compartments and bacterium-containing vacuoles. Our view is further corroborated by the finding that CD8+ T cells respon-

sive to *M. avium* antigens are present in mice infected with these bacilli (18).

Additional studies are required to determine if *M. avium*-containing vacuoles in DC are ruptured and if *M. avium*-infected DC are able to expand antigen-specific CD8$^+$ cytotoxic T cells in vitro, which may have important implications for immunotherapy in conjunction with chemotherapy for management of opportunistic infection.

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