Processing of Mycobacterium tuberculosis Bacilli by Human Monocytes for CD4+ αβ and γδ T Cells: Role of Particulate Antigen

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Mycobacterium tuberculosis readily activates both CD4+ and Vδ2+ γδ T cells. Despite similarity in function, these T-cell subsets differ in the antigens they recognize and the manners in which these antigens are presented by M. tuberculosis-infected monocytes. We investigated mechanisms of antigen processing of M. tuberculosis antigens to human CD4+ and γδ T cells by monocytes. Initial uptake of M. tuberculosis bacilli and subsequent processing were required for efficient presentation not only to CD4+ T cells but also to Vδ2+ γδ T cells. For γδ T cells, recognition of M. tuberculosis-infected monocytes was dependent on Vδ2+ T-cell-receptor expression. Recognition of M. tuberculosis antigens by CD4+ T cells was restricted by the class II major histocompatibility complex molecule IHLA-DR. Processing of M. tuberculosis bacilli for Vδ2+ γδ T cells was inhibitable by Brefeldin A, whereas processing of soluble mycobacterial antigens for γδ T cells was not sensitive to Brefeldin A. Processing of M. tuberculosis bacilli for CD4+ T cells was unaffected by Brefeldin A. Lysosomotropic agents such as chloroquine and ammonium chloride did not affect the processing of M. tuberculosis bacilli for CD4+ and γδ T cells. In contrast, both inhibitors blocked processing of soluble mycobacterial antigens for CD4+ T cells. Chloroquine and ammonium chloride insensitivity of processing of M. tuberculosis bacilli was not dependent on the viability of the bacteria, since processing of both formaldehyde-fixed dead bacteria and mycobacterial antigens covalently coupled to latex beads was chloroquine insensitive. Thus, the manner in which mycobacterial antigens were taken up by monocytes (particulate versus soluble) influenced the antigen processing pathway for CD4+ and γδ T cells.

Mycobacterium tuberculosis, the etiologic agent of human tuberculosis, is spread readily from person to person by inhalation of aerosolized mycobacteria (8). A hallmark of M. tuberculosis infection is the ability of most healthy individuals to control the infection by mounting an acquired immune response (37). In this process, CD4+ T cells, recognition of mycobacterial peptides in the context of class II major histocompatibility complex (MHC) molecules, which include secreted as well as somatic antigens, is restricted by the class II major histocompatibility complex molecule IHLA-DR. Processing of M. tuberculosis bacilli for CD4+ T cells was restricted by the class II major histocompatibility complex molecule IHLA-DR. Processing of M. tuberculosis bacilli for Vδ2+ γδ T cells was inhibitable by Brefeldin A, whereas processing of soluble mycobacterial antigens for γδ T cells was not sensitive to Brefeldin A. Despite similarities in function, these two T-cell subsets differ in the mycobacterial antigens recognized by their TCRs (17, 31, 32). Within mononuclear phagocytes, the TCR subsets activated by M. tuberculosis, recognize mycobacterial antigens in a non-MHC-restricted manner and the repertoire of antigens includes small phosphate-containing antigens such as TUBag’s (5, 9, 19, 22, 29, 36).

Both blood monocytes and alveolar macrophages infected with M. tuberculosis are efficient antigen-presenting cells for mycobacterial antigen-specific CD4+ and γδ T cells (1, 5). However, little is known about how M. tuberculosis-infected mononuclear phagocytes process antigens for these two T-cell subsets. M. tuberculosis bacilli are taken up by mononuclear phagocytes through a variety of surface receptors, including complement receptor 4, mannose receptor, and complement receptor 3 (17, 31, 32). Within mononuclear phagocytes, the mycobacteria reside within phagosomes and modulate the phagosome by preventing fusion with acidic lysosomal compartments (7). Although the vacuolar membranes surrounding...
the phagosome acquire endosomal markers, the vesicular proton ATPase is actively excluded, resulting in an elevated pH of 6.3 to 6.5 compared to the normal lysosomal pH of 4.5 (7, 35). The elevated pH in the phagosome does not appear to inhibit the ability of mycobacterial antigens to be processed and presented to CD4+ and Vδ2+ γδ T cells. This study was undertaken to gain insight into the mechanisms used by monocytes infected with live M. tuberculosis bacilli to process mycobacterial antigens for presentation to both CD4+ and γδ T cells.

MATERIALS AND METHODS

Chemical reagents and monoclonal antibodies. Chloroquine, ammonium chloride, cyclohexatin D, and Brefeldin A were purchased from Sigma (St. Louis, Mo.). Chloroquine and ammonium chloride were dissolved in phosphate-buffered saline, Phycocyanin (PE)-conjugated anti-CD11c-2 receptor alpha chain (IL-2Ro) (CD25-PE; Becton Dickinson, San Jose, Calif.), fluorescein isothiocyanate (FITC)-conjugated OKT-4 (CD4-FITC; Ortho Diagnostics, Raritan, N.J.), and FITC-conjugated TRC-1 (γδ-FITC; T Cell Sciences, Cambridge, Mass.) were purchased and used according to the manufacturers’ instructions with FITC- and PE-conjugated isotypic controls. Purified anti-human TCR-V62 antibody C448.15D was a gift from Simon R. Carding, University of Pennsylvania, Philadelphia. Purified L243 (anti-HLA-DR) was a gift from Cliff Harding. (Case Western Reserve University, Cleveland, Ohio).

Background studies. M. tuberculosis H37Ra was cultured in Middlebrook 7H9 with ADC enrichment, and frozen stocks were prepared as described previously (5, 15). Bacterial counts and viability were performed by light microscopy and by counting CFU on 7H10 medium. M. tuberculosis H37Ra stocks were tested periodically for viability and with an M. tuberculosis complex-specific DNA probe (Accucore; Gen-Probe, San Diego, Calif.) to ensure purity of the M. tuberculosis stocks. Before use in T-cell assays, mycobacteria were washed three times in RPMI 1640 and sonicated for 20 to 30 s to disrupt clumps. The viability was routinely more than 50%.

Formaldehyde-fixed M. tuberculosis was prepared by suspending bacilli in 1 ml of RPMI 1640 (10^9/ml) containing 1.5% (vol/vol) formaldehyde as previously described (20). Briefly, mycobacteria were incubated at room temperature for 2 h with constant mixing, pelleted, and washed three times. The cells were resuspended in 1 ml of RPMI 1640 and kept at 4°C. Viability was less than 1 CFU/ml.

Cytosolic antigens of M. tuberculosis H37Ra were prepared as previously described (37). Cytosolic mycobacterial antigens were coupled covalently to carboxylated latex beads by carbodiimide linkage (Polysciences, Inc., War- rington, Pa.) as per the manufacturer’s instructions. Briefly, 0.5 ml of a 2.5% suspension of carboxylated beads was washed with carbonate buffer followed by phosphate buffer (4). Dispersion of cytosolic mycobacterial antigens was achieved by incubation with 0.1 M ethane diamine tetraacetic acid for 30 min, and inhibitors were removed by washing. Monocytes were then washed and resuspended in 0.1 M ethane diamine tetraacetic acid (pH 7.2) for 20 min at room temperature and then washed (four times). Cells were incubated in tissue culture medium for 60 min to remove residual paraformaldehyde before use in proliferation assays. Before the cells were washed with 1 µCi of [3H]thymidine, 50 µl of supernatant was harvested to measure the levels of secreted cytokines. The levels of IFN-gamma were measured by enzyme-linked immunosorbent assay (Endogen, Cambridge, Mass.).

For cytokine uptake and processing, monocytes were incubated with whole M. tuberculosis (51 or 101 bacteria-to-cell ratio) for 4 h. Then cells were washed and fixed with paraformaldehyde as previously described (3). Briefly, cells were washed and fixed with paraformaldehyde for 1 min, followed by neutralization with 0.15 M glycine (pH 7.2) for 20 min at room temperature and then washing (four times). Cells were incubated in tissue culture medium for 60 min to remove residual paraformaldehyde before use in proliferation assays. After the cells were washed with 1 µCi of [3H]thymidine, 50 µl of supernatant was harvested to measure the levels of secreted cytokines.

RESULTS

Requirement for phagocytosis and processing of M. tuberculosis bacilli by monocytes for CD4+ and γδ T cells. In earlier studies, we demonstrated that live M. tuberculosis bacilli readily activate peripheral blood γδ T cells from healthy tuberculin-positive persons. Activation of T cells by live M. tuberculosis is not restricted to γδ T cells but also results in activation of CD4+ T cells. A representative experiment is shown in Fig. 1, in which IL-2Ro (CD25) expression on CD4+ and γδ T cells was measured after stimulation of PBMC with live M. tuberculosis bacilli. The activation of these two T-cell subsets by M. tuberculosis antigens is dependent on antigen-presenting cells, and both monocytes are efficient APC for both CD4+ and γδ T cells. We have used the activation of peripheral blood CD4+ and γδ T cells by M. tuberculosis bacilli as a means to derive M. tuberculosis bacilli cell line used in biweekly stimulation with mycobacterial antigens, irradiated PBMC, and recombinant IL-2. CD4+ T cell lines were maintained on autologous PBMC as APC, and generally γδ T cell lines were stimulated with HLA-mismatched PBMC. Mycobacterial antigen-specific T-cell lines stimulated in vitro two to three times and maintained for 8 weeks were considered short-term lines and were derived from seven donors (seven lines for CD4+ T cells, and four lines for γδ T cells). Tcels (n = 4 for CD4+ T cells, n = 2 for γδ T cells) maintained for more than 12 weeks were considered long-term lines. Most experiments shown were performed with long-term T-cell lines; however, results were validated with both short-term and long-term lines. Pursuit of the phenotype of the T-cell line was monitored by flow cytometry.

Proliferation assays. CD4+ and γδ T cells (2 × 10^9 to 2.5 × 10^9 per 200-µl well) were cocultured with 5 × 10^5 monocytes per well as APC for 72 h in 96-well plates. Cells were washed with 1 µCi of [3H]thymidine (ICN, Costa Mesa, Calif.) for 12 to 16 h before being harvested on glass fiber filters. [3H]thymidine incorporation was measured by liquid scintillation counting and expressed as counts per minute.

Statistical analysis. Statistical analysis was determined by paired Student’s t test, and a P of <0.05 was considered significant.
tuberculosis antigen-specific CD4\(^+\) and γδ T-cell lines for the antigen-processing experiments described below. Whereas αβ TCR usage and mycobacterial antigen recognition by CD4\(^+\) T cells are characterized by marked diversity, γδ T-cell activation by mycobacterial antigens is limited predominantly to T cells expressing TCR consisting of V\(_g\)9 and V\(_d\)2 chains. As shown in Fig. 2B, activation of γδ T cells was inhibited by MAb C448.15D, specific for the V\(_d\)2 chain of γδ TCR, indicating that activation of γδ T cells by live-M. tuberculosis-infected monocytes was dependent on V\(_d\)2 expression. Recognition of M. tuberculosis by CD4 T cells was blocked by anti-HLA-DR antibody (Fig. 2A); in contrast, recognition of M. tuberculosis by γδ T cells is not restricted by MHC molecules (5).

Next, we determined if phagocytosis and processing by monocytes of M. tuberculosis bacilli were required for presentation of antigens to CD4\(^+\) and γδ T-cell lines. Monocytes were pretreated with cytochalasin D, an inhibitor of phagocytosis by inhibiting actin polymerization, for 30 min before M. tuberculosis-infected monocytes were dependent on V\(_d\)2 expression. Recognition of M. tuberculosis by CD4 T cells was blocked by anti-HLA-DR antibody (Fig. 2A); in contrast, recognition of M. tuberculosis by γδ T cells is not restricted by MHC molecules (5).

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Cytochalasin D pretreatment did not inhibit the ability of monocytes to present soluble mycobacterial antigens to both T-cell subsets, indicating that cytochalasin D treatment did not affect APC function of monocytes (41,921 cpm).

Fixation of monocytes with paraformaldehyde before exposure to M. tuberculosis prevented processing and presentation of mycobacterial antigens to both CD4\(^+\) and γδ T cells, whereas monocytes fixed with paraformaldehyde after infection with M. tuberculosis were able to activate both T-cell subsets (Fig. 4). In addition, we measured IFN-γ release in response to fixed APC by CD4 and γδ T-cell lines. IFN-γ results were similar to proliferation results. Fixing the monocytes before pulsing them with bacteria did not stimulate CD4 and γδ T cells to make detectable levels of IFN-γ, while fixing the monocytes after infection with M. tuberculosis induced IFN-γ production by CD4 T cells (949 pg/ml) and γδ T cells (354 pg/ml). Consistent with the results of the proliferation experiments, IFN-γ levels in response to fixed APC were reduced 50% in comparison with those in response to unfixed APC.

Inhibition by Brefeldin A of mycobacterial antigen processing for γδ but not CD4\(^+\) T cells. The above-described experiments demonstrated that initial uptake by phagocytosis and antigen processing were required to present antigens from M. tuberculosis antigen-specific CD4\(^+\) and γδ T-cell lines for the antigen-processing experiments described below. Whereas αβ TCR usage and mycobacterial antigen recognition by CD4\(^+\) T cells are characterized by marked diversity, γδ T-cell activation by mycobacterial antigens is limited predominantly to T cells expressing TCR consisting of V\(_g\)9 and V\(_d\)2 chains. As shown in Fig. 2B, activation of γδ T cells was inhibited by MAb C448.15D, specific for the V\(_d\)2 chain of γδ TCR, indicating that activation of γδ T cells by live-M. tuberculosis-infected monocytes was dependent on V\(_d\)2 expression. Recognition of M. tuberculosis by CD4 T cells was blocked by anti-HLA-DR antibody (Fig. 2A); in contrast, recognition of M. tuberculosis by γδ T cells is not restricted by MHC molecules (5).

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tuberculosis bacilli by monocytes to CD4⁺ and γδ T cells. To further characterize the antigen-processing pathways of M. tuberculosis-infected monocytes, the effects of inhibitors of antigen processing were tested in cytotoxicity assays with CD4⁺ and γδ T cells as cytotoxic T lymphocytes (CTL). Processing of M. tuberculosis bacilli by monocytes was inhibited by Brefeldin A for γδ T cells. The results of two representative experiments of five are shown in Fig. 5A and C. Processing for CD4⁺ T cells (Fig. 5B) was not inhibited by Brefeldin A in the CTL assay (n = 4, P > 0.375). The same M. tuberculosis-infected monocytes were used in CTL assays with both CD4⁺ and γδ T cells (Fig. 5A and B), clearly establishing the differential effects of Brefeldin A on processing of M. tuberculosis bacilli for these two T-cell subsets (inhibition for γδ T cells was 53 to 60% in five experiments; P < 0.025). Processing for γδ T cells (and for CD4⁺ T cells [data not shown]) of soluble cytosolic antigens of M. tuberculosis by monocytes was not sensitive to Brefeldin A treatment (5 μg/ml), in contrast to the findings with intact bacilli (Fig. 5D) (n = 5, P > 0.4). In these experiments, monocytes were exposed first to either live M. tuberculosis or soluble mycobacterial antigens and second to treatment with Brefeldin A for 2 h and then washed extensively before being tested in the CTL assay. No further antigens were added during the CTL assay, indicating that the ligand(s) recognized by γδ T cells was stably expressed on the surfaces of antigen-pulsed monocytes. Increasing the concentration of Brefeldin A to 20 μg/ml did not change the differential effects on antigen processing for γδ and CD4⁺ T cells (data not shown). These results indicated that in M. tuberculosis-infected monocytes, Brefeldin A inhibited ongoing processing for γδ T cells of antigens originating from the bacilli but not for CD4⁺ T cells.

The Vγ9⁺ and Vδ2⁺ γδ T-cell lines in this study did respond to isopentenyl pyrophosphate (IPP; 25 μg/ml). However, in contrast to M. tuberculosis bacilli or soluble mycobacterial antigens, IPP could not be stably pulsed onto monocytes for use in CTL assays but needed to be added and present continuously during a 4-h CTL assay (data not shown).

Inability of lysosomotropic agents to inhibit processing of M. tuberculosis bacilli for CD4⁺ and γδ T cells. Since M. tuberculosis bacilli reside largely within the phagosomes of mononuclear phagocytes and are able to inhibit phagosomal acidification, the effects of lysomotropic agents on antigen processing of M. tuberculosis bacilli for γδ and CD4⁺ T cells were tested. As shown in Fig. 6A and C, treatment of M. tuberculosis-infected monocytes with chloroquine did not affect ongoing

![Image](http://iai.asm.org/)
antigen processing for CD4\(^+\) T cells. In fact, in three of six experiments chloroquine appeared to enhance processing of M. tuberculosis, as was reflected in increased cytotoxicity (Fig. 6A and 6C) \((n = 6, P > 0.375)\). Processing of M. tuberculosis bacilli for gd T cells was not affected by chloroquine (data not shown).

The lack of effect of chloroquine on M. tuberculosis processing was observed for concentrations ranging from 40 to 2,000 \(\mu\text{M}\), with 400 \(\mu\text{M}\) being used as the optimal concentration. The insensitivity to chloroquine was not dependent on the load of M. tuberculosis bacilli when M. tuberculosis-to-monocyte ratios were varied from 1:1 to 30:1 (data not shown). Antigen processing of soluble mycobacterial antigens for CD4\(^+\) T cells was inhibited by chloroquine, serving as a control for the effect of chloroquine (Fig. 6B and D) \((n = 6, P < 0.025)\). The inability of chloroquine to inhibit processing of live M. tuberculosis for CD4\(^+\) T cells suggested that processing for class II MHC molecules of M. tuberculosis antigens from live bacilli

![FIG. 4. Requirement for uptake and processing of M. tuberculosis bacilli for presentation to both CD4\(^+\) and \(\gamma\delta\) T cells. Monocytes (MO) were fixed with paraformaldehyde either before (MO→FIXED) or after [(MO+MTB)→FIXED] a 120-min incubation with live M. tuberculosis (MTB). Fixed monocytes then were added to a proliferation assay with CD4\(^+\) (A) and \(\gamma\delta\) (B) T cells. Results are means and standard deviations of triplicate wells and are representative of four experiments.](https://iai.asm.org/)

![FIG. 5. Differential effects of Brefeldin A on antigen processing of M. tuberculosis bacilli for \(\gamma\delta\) and CD4\(^+\) T cells. Monocytes incubated for 12 h with either M. tuberculosis (MTB) (A, B, and C) or cytosolic mycobacterial antigens (Ag) (D) were treated with Brefeldin A (BFA) for 120 min before serving as targets in a CTL assay with either \(\gamma\delta\) T-cell lines (A, C, and D) or CD4\(^+\) T cell lines (B). In experiment 1 (EXP 1), the same monocyte targets were used for both CD4\(^+\) and \(\gamma\delta\) T-cell lines, with the \(\gamma\delta\) T-cell lines being derived from an HLA-mismatched donor. Results are representative of four experiments. E:T ratio, effector-to-target ratio.](https://iai.asm.org/)
differed from processing of soluble mycobacterial antigen. However, in these experiments chloroquine was added to monocytes already infected with *M. tuberculosis* or pulsed with soluble antigens, and thus the effect of chloroquine was on ongoing antigen processing. Thus, we determined the effect of chloroquine treatment on monocytes as they initiate the processing of mycobacterial antigens for CD4$^+$ T cells. In these experiments, monocytes were treated with chloroquine during a 4-h exposure to *M. tuberculosis* bacilli. As shown in the results of a representative experiment in Fig. 7, chloroquine did not inhibit processing of antigens of live *M. tuberculosis* bacilli ($n=4$, $P>0.375$) but did inhibit processing of soluble antigens (data not shown). To ensure that insensitivity of processing of *M. tuberculosis* bacilli for CD4$^+$ T cells and γδ T cells was not restricted to chloroquine, additional experiments with an alternative lysomotropic agent, ammonium chloride (NH$_4$Cl), were performed. As shown in Fig. 8, treatment of monocytes with ammonium chloride at the time of initial uptake did not inhibit subsequent processing of *M. tuberculosis* bacilli for CD4$^+$ T cells (Fig. 8A) ($n=3$, $P>0.375$) but did inhibit processing of soluble antigen (Fig. 8B) ($n=3$, $P<0.05$), indicating that processing of *M. tuberculosis* bacilli was resistant to two different lysomotropic agents.

**Chloroquine insensitivity of processing of particulate mycobacterial antigens for CD4$^+$ T cells.** To determine if chloroquine insensitivity of antigen processing for CD4$^+$ T cells was unique to live *M. tuberculosis* bacilli or due to the particulate nature of the antigen, experiments were performed with cytosolic antigens of *M. tuberculosis* covalently linked to latex beads. As shown in Fig. 9A and C, these particulate antigen preparations were also found to be chloroquine insensitive ($n=3$, $P>0.3$), whereas processing of soluble cytosolic antigens for CD4$^+$ T cells remained inhibitable with chloroquine in the same experiment (Fig. 9B) ($P<0.005$). When formaldehyde and dead *M. tuberculosis* bacilli were used, again no inhibition by chloroquine was observed, whereas soluble antigens were inhibited (data not shown). These findings suggested that it is the particulate nature of mycobacterial anti-
M. tuberculosis bacilli was not dependent on the viability of the bacteria, since both formaldehyde-fixed dead bacteria and mycobacterial antigens covalently coupled to latex beads were chloroquine insensitive. Thus, the particulate nature of mycobacterial antigens may have a role in determining insensitivity to agents.

In the last few years, increasing evidence suggests that M. tuberculosis-activated V62+ γδ T cells are predominantly activated by small phosphate-containing molecules. A number of these antigens have been identified and include four TUBag’s isolated from M. tuberculosis, which are small (≤500 Da) phosphorylated molecules which also contain nucleotide (9). Others have described IPP and related prenyl phosphates as possible antigens for V62+ T cells (36). These phosphate ligands are not secreted by mycobacteria belonging to the M. tuberculosis group. Instead, they remain associated with the bacterial cell mass (10). Studies with TUBag’s and synthetic IPP determined that these small antigens could be presented by fixed APC without a requirement for processing (22, 24). However, these small antigens were not stably associated with APC since antigen had to be present continuously during the assay and could not be pulsed onto APC (10). These studies also determined that T-cell–APC contact was required for activation of V62+ γδ T cells.

In contrast, our studies with M. tuberculosis bacilli indicated that antigens for V62+ γδ T cells are processed and remain stably associated on the surfaces of monocytes. This conclusion was supported by three lines of evidence. First, monocytes fixed before addition of M. tuberculosis bacilli were unable to activate V62+ γδ T cells whereas those fixed after infection were able to present antigen. Second, monocytes infected with M. tuberculosis or pulsed with soluble mycobacterial antigens could be washed extensively and still present antigen to V62+ γδ T cells, indicating that antigens for V62+ γδ T cells remained stably associated with monocyte surfaces. The γδ-T-cell lines used in our study did react to synthetic small phosphate-containing antigens such as IPP. However, IPP was not stably associated on monocytes for recognition by γδ T cells and was readily removed by washing. In contrast, soluble mycobacterial antigen and live M. tuberculosis bacilli were stably pulsed onto monocytes and extensive washing did not remove antigen for V62+ γδ T cells. Third, Brefeldin A could inhibit processing of M. tuberculosis antigens for V62+ γδ T cells. Brefeldin A inhibits transport from the endoplasmic reticulum to the trans-Golgi network, thus suggesting either that in M. tuberculosis-infected monocytes, antigen(s) for γδ T cells becomes associated with molecules migrating from the endoplasmic reticulum to the trans-Golgi network or that M. tuberculosis antigens for γδ T cells require transport through the endoplasmic reticulum and trans-Golgi network to arrive on the surfaces of monocytes. These results are consistent with a model in which small phosphate antigens or epitopes of M. tuberculosis are associated with a carrier molecule which requires processing and allows phosphate antigens or epitopes to remain stably associated on the surfaces of APC. Our observation that M. tuberculosis bacilli contain a 10- to 14-kDa cytosolic antigen which stimulates V62+ γδ T cells is consistent with this model (4). Further supporting evidence for a carrier molecule was provided by the ability of cytosolic antigens covalently linked to latex beads to stimulate V62+ γδ T cells with monocytes as APC (data not shown).

In contrast to the poorly understood processing of antigens for γδ T cells, the cellular pathways for processing of soluble antigens for presentation by class II MHC molecules to CD4+ T cells are much better characterized (reviewed in reference

### DISCUSSION

Monocytes infected with M. tuberculosis are efficient antigen-presenting cells for mycobacterial antigen-specific CD4+ and γδ T cells from healthy tuberculin skin test-positive persons (25). The results of our studies indicate that there are a number of unique features to the mechanisms used by human monocytes to process and present antigens originating from M. tuberculosis-containing phagosomes to both of these T-cells subsets. First, uptake of M. tuberculosis bacilli by phagocytosis and subsequent processing were required for antigen presentation to both CD4+ and γδ T cells. Second, the recognition of M. tuberculosis-infected monocytes by γδ T cells was dependent on V62+ TCR and by CD4+ T cells on class II MHC molecules. Processing of antigens for V62+ γδ T cells was inhibited by Brefeldin A. In contrast, processing of M. tuberculosis for CD4+ T cells was unaffected by Brefeldin A. Third, lysomotropic agents such as chloroquine and ammonium chloride did not affect the processing of M. tuberculosis bacilli for CD4+ and γδ T cells. This was true both when inhibitors were added during initial uptake and short-term processing of M. tuberculosis bacilli and when lysomotropic agents were added to ongoing antigen processing. In contrast, both inhibitors were able to block the processing of soluble mycobacterial antigens for CD4+ T cells in both situations. Fourth, the chloroquine and ammonium chloride insensitivity of processing of M. tuberculosis bacilli was not dependent on the viability of the bacteria, since both formaldehyde-fixed dead bacteria and mycobacterial antigens covalently coupled to latex beads were chloroquine insensitive. Thus, the particulate nature of mycobacterial antigens may have a role in determining insensitivity to agents.
11). Antigens are internalized by endocytosis or phagocytosis and concentrated within endosomes. As endosomes mature and fuse with lysosomes, proteases break down protein antigens into peptide fragments and the pH progressively decreases. Class II MHC molecules are present in late endosomal and early lysosomal compartments, where antigen-derived peptides bind to class II MHC molecules. The processing of particulate antigens (e.g., bacteria) is less-well-understood, and the site where mycobacterial peptides are loaded on class II MHC molecules in macrophages is unknown. After phagocytosis, \textit{M. tuberculosis} bacilli generally remain within phagosomes and class II MHC molecules can be found in these phagosomes (7). \textit{M. tuberculosis}-infected monocytes readily process and present antigens to CD4$^+$ T cells. Our finding that processing of \textit{M. tuberculosis} bacilli is resistant to lysosomotropic agents is consistent with the possibility that mycobacterial antigens become associated with class II MHC molecules within phagosomes.

Our finding that processing of \textit{M. tuberculosis} bacilli is resistant to lysosomotropic agents is consistent with the possibility that mycobacterial antigens become associated with class II MHC molecules within phagosomes. Chloroquine-resistant processing of \textit{M. tuberculosis}, however, is not unique to live bacteria, since processing of particulate mycobacterial antigens for CD4$^+$ T cells also is chloroquine resistant. Thus, processing of particulate mycobacterial antigens by monocytes may not depend on acidification of the phago-lysosomal compartment. Our studies were performed with live \textit{M. tuberculosis}, formaldehyde-fixed bacteria, and cytosolic antigens of \textit{M. tuberculosis} linked to latex beads. These are complex antigen preparations and include not only a large number of different proteins but also nonprotein constituents such as lipoarabinomannan, phosphatidyl-myoinositol mannosides and other phospholipids, and complex carbohydrates, which may influence antigen processing in endosomal compartments. Traffic of lipoarabinomannan out of phagosomes has been demonstrated, supporting the concept of dynamic interactions between \textit{M. tuberculosis} phagosomes and endosomal compartments (38). Chloroquine-resistant processing of particulate mycobacterial antigens may be unique to mycobacteria, since the original studies of inhibition of antigen processing for class II MHC molecules by agents were performed with particulate heat-killed \textit{Listeria monocytogenes} and murine peritoneal macrophages (39). Alternatively, it is possible that human monocytes differ from murine macrophages in how they process particulate antigens for class II MHC molecules. Based on studies with human macrophages chronically infected with \textit{M. bovis} BCG, it has been suggested that mycobacteria can reside within a compartment outside the route of normal antigen processing for CD4$^+$ T cells (28). This result is consistent with our findings that particulate mycobacterial antigens may have unique antigen-processing requirements.

The antigen-processing mechanisms used by \textit{M. tuberculosis}-infected mononuclear phagocytes are critical in the recruitment of T-cell subsets and in the determination of the antigen repertoire recognized by CD4$^+$ and $\gamma\delta$ T cells. Our study indicates that there are distinct pathways for the processing by human monocytes of antigens emanating from \textit{M. tuberculosis}-containing phagosomes for CD4$^+$ and $\gamma\delta$ T cells. In addition, the manner in which the antigen is present within monocytes (particulate versus soluble) influences the antigen-processing mechanisms. Further studies of the antigen processing of \textit{M. tuberculosis} bacilli by macrophages is necessary to understand the regulation of T-cell-subset activation in the protective immune response to \textit{M. tuberculosis}, an understanding of which is necessary for the design of improved vaccines and immunotherapies for tuberculosis.
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