The Human CD1-Restricted T Cell Repertoire Is Limited to Cross-Reactive Antigens: Implications for Host Responses against Immunologically Related Pathogens

Peter A. Sieling, Jordi B. Torrelles, Steffen Stenger, Woosin Chung, Anne E. Burdick, Thomas H. Rea, Patrick J. Brennan, John T. Belisle, Steven A. Porcelli and Robert L. Modlin

*J Immunol* 2005; 174:2637-2644; doi: 10.4049/jimmunol.174.5.2637

http://www.jimmunol.org/content/174/5/2637
The Human CD1-Restricted T Cell Repertoire Is Limited to Cross-Reactive Antigens: Implications for Host Responses against Immunologically Related Pathogens

Peter A. Sieling,* Jordi B. Torrelles,§ Steffen Stenger,‖ Woosin Chung,* Anne E. Burdick,¶ Thomas H. Rea,* Patrick J. Brennan,§ John T. Belisle,§ Steven A. Porcelli,** and Robert L. Modlin*†‡

The repertoires of CD1- and MHC-restricted T cells are complementary, permitting the immune recognition of both lipid and peptide Ags, respectively. To compare the breadth of the CD1-restricted and MHC-restricted T cell repertoires, we evaluated T cell responses against lipid and peptide Ags of mycobacteria in leprosy, comparing tuberculoid patients, who are able to restrict the pathogen, and lepromatous patients, who have disseminated infection. The striking finding was that in lepromatous leprosy, T cells did not efficiently recognize lipid Ags from the leprosy pathogen, Mycobacterium leprae, or the related species, Mycobacterium tuberculosis, yet were able to efficiently recognize peptide Ags from M. tuberculosis, but not M. leprae. To identify a mechanism for T cell unresponsiveness against mycobacterial lipid Ags in lepromatous patients, we used T cell clones to probe the species specificity of the Ags recognized. We found that the majority of M. leprae-reactive CD1-restricted T cell clones (92%) were cross-reactive for multiple mycobacterial species, whereas the majority of M. leprae-reactive MHC-restricted T cells were species specific (66%), with a limited number of T cell clones cross-reactive (34%) with M. tuberculosis. In comparison with the MHC class II-restricted T cell repertoire, the CD1-restricted T cell repertoire is limited to recognition of cross-reactive Ags, imparting a distinct role in the host response to immunologically related pathogens. The Journal of Immunology, 2005, 174: 2637–2644.

*Division of Dermatology, †Department of Microbiology and Immunology, and *Molecular Biology Institute, David Geffen School of Medicine at University of California-Los Angeles, Los Angeles, CA 90095; ‡Department of Microbiology, Immunology, and Pathology College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; †Institute for Clinical Microbiology, Immunology, and Hygiene, Universitat Erlangen, Erlangen, Germany; †Department of Dermatology and Cutaneous Surgery, University of Miami, Miami, FL 33101; ‡Section of Dermatology, Keck School of Medicine at University of Southern California, Los Angeles, CA 90033; and **Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461

Received for publication April 22, 2004. Accepted for publication December 2, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by the National Institutes of Health (Grants AI40312 and AI22553 (to R.L.M.) and Grants AI48589 and AI48933 (to S.A.P.) and received material support through National Institutes of Health Contracts N01-AI-75320 (to J.T.B.) and N01-AI-25469 (to P.J.B.). Additional financial support was provided by the United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases (to R.L.M.) and a Clinical Scientist Award in Translational Research from the Burroughs-Wellcome Fund (to S.A.P.).

Address correspondence and reprint requests to Dr. Peter A. Sieling, Division of Dermatology/Department of Medicine David Geffen School of Medicine at University of California-Los Angeles, 52-121 CHS, 10833 Le Conte Avenue, Los Angeles, CA 90095. E-mail address: psieling@mednet.ucla.edu

The Ag-presenting pathways of the MHC and MHC-like proteins, including CD1, complement each other and serve to shape the T cell repertoire against microbial infection. Whereas MHC class I and class II engage microbial Ags in distinct subcellular compartments, MHC and CD1 present distinct antigenic structures to T cells. MHC molecules present peptide Ags, in contrast to CD1 molecules, which present lipid Ags (1–5). The structures of lipid Ags are likely to be more conserved between microbial species compared with peptides because lipids are essential to the integrity of the microorganisms’ cellular envelope. The conserved structures of lipid Ags raise the question of how the CD1-restricted T cell repertoire is shaped during the course of infection.

The CD1 family of proteins is segregated into two subgroups based on sequence similarity. Group 1 proteins, CD1a, CD1b, and CD1c, are much more closely related to one another than they are to CD1d. Group 2 proteins include human CD1d and murine CD1. Group 1 CD1-restricted T cells are activated directly by microbial lipid Ags and may contribute to host defense against infection (1, 2, 6–8), whereas group 2 CD1-restricted T cells probably do not respond directly to microbial ligands (9) but have a regulatory function (10, 11).

We investigated the group 1 CD1-restricted T cell repertoire in the immune response to infection using leprosy as a model. Leprosy presents as a spectrum in which clinical disease correlates with different levels of T cell responsiveness to Mycobacterium leprae (12), the causative pathogen. At one pole are patients with strong cell-mediated immunity to M. leprae and a localized form of the disease, which constitutes tuberculoid leprosy. At the opposite pole are patients with lepromatous leprosy, who lack effective cell-mediated immunity and suffer from a more disseminated form of the disease. The existence of this spectrum provides the opportunity to assess immunoregulatory mechanisms that may operate in vivo in humans to determine the ultimate outcome of the immune response to infection. Our previous studies have indicated that CD1-restricted T cells contribute to host defense against leprosy infection (2, 6, 13). To better characterize the contribution of the CD1-restricted T cell response to infection, we compared the repertoire of CD1- and MHC-restricted T cells in the context of human leprosy.

Materials and Methods

Patients and clinical specimens

Leprosy patients were recruited on a volunteer basis from the ambulatory population seen at Hansen’s Disease Clinics at Los Angeles County/University of Southern California and University of Miami Medical Centers. Clinical classification of patients with symptomatic M. leprae infection was performed according to the criteria of Ridley and Jopling (12). Patients
presenting with de novo tuberculoid leprosy or exhibiting reversal reactions were defined as T-Lep, and those presenting with poliar lepromatous either with or without erythema nodosum lepromatous reactions were defined as L-Lep. Additional information on the patients is detailed in Table 1. Blood samples for isolation of PBMC were obtained by venipuncture from leprosy patients and healthy volunteers after obtaining their informed consent. PBMC were isolated using Ficoll-Hypaque gradient centrifugation (Ficoll-Paque; Pharmacia Biotech).

### Ags and Abs

Extracts of *M. leprae* and *M. tuberculosis* (strain H37Ra (Difco) and clinical isolate TSU20 (14)) were prepared by probe sonication as previously described (15). Lipid preparations of mycobacterial sonicates were prepared by extraction with chloroform/methanol (2/1) (1). The following Abs were used for flow cytometry studies: OKT6 (anti-CD1a) (16), BCD1b3.1 (anti-CD1b) (17), F10/21A3 (anti-CD1c) (18), and appropriate isotype controls. To degrade protein Ags, sonicated *M. leprae* was treated with proteinase K (0.7 mg/ml; Roche) for 30 min at 60°C, and the enzyme was heat-inactivated for 10 min at 70°C. Control samples were incubated with proteinase K that was heat-inactivated before mixing with the mycobacterial extract.

### In vitro culture of CD1-expressing monocyte-derived dendritic cells

CD1+ monocyte-derived dendritic cells were generated in vitro with a combination of recombinant human GM-CSF (200 U/ml) and recombinant human IL-4 (100 U/ml) as previously described (19, 20). Cells were harvested using incubation in PBS/0.5 mM EDTA to detach adherent cells, then were analyzed by flow cytometry using CD1-specific mAbs (19) or irradiated (5000 rad) and used as APCs.

### T cell lines and proliferation assays

T cell lines were derived from leprosy lesions and blood from healthy donors as previously defined (2, 21). Briefly, cells were extracted from lesions with a tissue sieve, and lymphocytes were isolated by density gradient centrifugation. T cell lines were initiated in the presence of irradiated autologous PBMCs and IL-2, followed by culture with HLA-DR-matched APCs or irradiated CD1+ APCs (19). T cell lines were maintained by serial antigenic stimulation in rIL-2 (1 nM; Chiron Diagnostics)-supplemented medium. Heterologous irradiated PBMCs and PHA were used to propagate T cell lines and to generate clones using limiting dilution (21). For measurement of Ag-specific proliferation, T cells (1 × 10^5) were cultured with varying numbers (usually 1 × 10^4) of irradiated (5000 rad) HLA-DR-matched or heterologous CD1+ APC in culture medium (0.2 ml) in the presence or the absence of bacterial Ags for 3 days in microtiter wells (in triplicate) at 37°C in a 7% CO2 incubator. Cells were pulsed with [H]thymidine (1 μCi/well; ICN Biomedicals) and harvested 4–6 h later for liquid scintillation counting. To determine CD1 restriction of the T cell lines, neutralizing CD1 Abs were added 30 min before the addition of T cells. To examine their role, CD8 T cells were depleted using mouse anti-human-CD8 beads (Dynal Biotech). Cytokine release from T cells was measured by ELISA after stimulation with CD1-positive APCs and Ag or medium for 24 h. IFN-γ ELISA (BD Pharmingen) was performed according to the instructions of the manufacturers.

### Measurement of cytokine-producing cells by ELISPOT

The frequencies of cytokine-producing cells were evaluated using an ELISPOT method. PBMC were isolated by density gradient centrifugation. Monocytes were enriched by adherence (2 h, 37°C in RPMI 1640 supplemented with 10% FBS), and nonadherent cells were removed and frozen to be tested later for cytokine production. Dendritic cells were derived from adherent cells using GM-CSF and IL-4 as described above. Dendritic cells were harvested, irradiated (5000 rad), and cultured (1 × 10^4) with nonadherent autologous cells (1 × 10^5–200 μl) in the presence or the absence of mycobacterial extracts (*M. leprae*, *M. tuberculosis*; 10 μg/ml) or PHA (2 μg/ml) for 24 h. Cells were transferred to ELISPOT plates (Cellular Technology) that had been previously coated with anti-cytokine Abs (mouse anti-human IFN-γ and IL-10 (R&D Systems); mouse anti-human IL-4 (BD Pharmingen)) and incubated for another 24 h. Cells were removed from the plate, and a biotinylated detecting Ab was added (goat anti-human IFN-γ and IL-10 (R&D Systems); rat anti-human IL-4 (BD Pharmingen)) for 1 h. Detecting Ab was removed, and a streptavidin alkaline phosphatase (Pierce) was added to the plate for 1 h. To visualize the cytokine-producing cells, substrate (5-bromo-4-chloro-3-indolyl-phosphate/NBT; Kirkegaard & Perry Laboratories) was added, and the plates were incubated in the dark for 1 h. ELISPOT plates were digitally scanned on an ImmunoSpot Image Analyzer (Cellular Technology) in the University of California-Los Angeles Immunology Core laboratory.

### Statistical comparisons

The Mann-Whitney U test was applied to compare the levels of IFN-γ-producing cells between patients at either pole of the leprosy spectrum. Nonparametric methods were used because the data were not normally distributed. A value of *p* < 0.05 was considered significant.

### Results

**CD1-restricted T cells detectable in peripheral blood of leprosy patients**

CD1-restricted T cells recognize lipid and glycolipid Ags from the cellular envelope of mycobacteria (1–3). Therefore, to enrich for CD1 Ags, lipid extracts of *M. leprae* and *M. tuberculosis* were prepared using organic solvents. The presence of CD1 glycolipid Ags in lipid extracts was evaluated by examining the T cell responses of established CD1-restricted T cells (6, 22). Lipid extracts from both *M. leprae* and *M. tuberculosis* (Fig. 1A) stimulated CD1-restricted T cell lines in a dose-dependent manner, indicating that the lipid extracts contained CD1 glycolipid Ags.

To determine the frequency of CD1-restricted T cells in the peripheral blood of leprosy patients, an IFN-γ ELISPOT method was established using the lipid extracts of mycobacteria. We measured IFN-γ by ELISPOT because 1) IFN-γ has been shown to contribute to immune protection against mycobacterial infection (23, 24); 2) CD1-restricted T cells from the lesions of leprosy patients produce IFN-γ (2); 3) ELISPOT is a very sensitive method to detect the frequency of Ag-reactive cells from within a population of lymphocytes with multiple specificities (25); and 4) IFN-γ ELISPOT analysis has previously been used as a means to measure the CD1-restricted T cell responses in tuberculosis patients (26). PBMC of tuberculoid leprosy patients produced IFN-γ in response to lipid extracts of *M. leprae*, and the responses were inhibited 50–100% by neutralizing Abs to CD1a and CD1b (Fig. 1B). Interestingly, Abs to CD1c did not inhibit IFN-γ-producing cells. CD1c Ags are glycolipids as are CD1b (27), although it is possible that the frequency of IFN-γ-producing T cells to CD1c Ags is too low to detect using the ELISPOT method. Alternatively, CD1c-restricted T cells may be skewed toward recognition of self-Ags (28). The data indicate that lipid extracts of mycobacteria activate CD1-restricted T cell responses from the blood of leprosy patients and that CD1 Ags are the predominant species in the lipid extracts. Conversely, peptide Ags are the predominant species in the total mycobacterial extracts because the T cell response to total extract is neutralized by protease treatment (Fig. 1C).

<table>
<thead>
<tr>
<th>Table 1. Patient characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient Classification</strong></td>
</tr>
<tr>
<td><strong>Number</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td><strong>Age in years (mean/median)</strong></td>
</tr>
<tr>
<td><strong>Age range (years)</strong></td>
</tr>
<tr>
<td><strong>Time since treatment began</strong></td>
</tr>
<tr>
<td><strong>Mean/median (years)</strong></td>
</tr>
</tbody>
</table>

Gender 16 male, 7 female 21 male, 7 female
Age in years (mean/median) 53.6/53 49.2/49
Age range (years) 22–79 23–74
Number 23 28
Time since treatment began 9.8/9 17.4/19
Mean/median (years) 0.06–25 3–31
FIGURE 1. CD1-restricted T cells detectable in the peripheral blood of leprosy patients. A, CD1-restricted T cell lines respond to lipid extracts of mycobacteria. T cell lines were cultured with monocyte-derived dendritic cells in the presence of lipid extracts of M. leprae (left panel) or M. tuberculosis (right panel). T cell activation was measured by IFN-γ production from triplicate cultures. B, Lipid extracts of mycobacteria stimulate CD1-restricted T cell responses from leprosy patients. Monocyte-derived dendritic cells and T cells from the autologous donor were cultured in the presence of M. leprae lipid extract. Neutralizing Abs to CD1 isoforms were added to evaluate the level of CD1 Ag presentation of the lipid extracts. IFN-γ-producing cells were measured using ELISPOT. C, T cell responses to M. leprae total extracts are predominantly peptide specific. T cells and autologous dendritic cells from T-Lep patients were cultured with M. leprae total extract treated with proteinase K or heat-inactivated enzyme. IFN-γ production was measured using ELISPOT. Values are expressed as the mean ± SEM of triplicate cultures.

Reduced frequency of mycobacteria-reactive, CD1-restricted T cells in patients with disseminated leprosy infection

Numerous efforts on our part to derive CD1-restricted T cells against mycobacteria from patients with disseminated leprosy infection have been unsuccessful. This together with our earlier finding that CD1+ dendritic cells are lower in lesions of lepromatous patients (13) lead us to hypothesize that CD1-restricted T cell responses are reduced in lepromatous patients. To test this hypothesis, the frequency of IFN-γ-producing cells from the blood of leprosy patients in response to whole and lipid-enriched mycobacterial extracts was measured using ELISPOT. The frequency of IFN-γ-producing cells was higher in the blood of tuberculoid patients in response to whole extracts of M. leprae (mean ± SEM, 16.4 ± 2.9 IFN-γ-producing cells/10³; n = 21; Fig. 2A) compared with lepromatous patients (8.3 ± 1.9; n = 20; p < 0.05). However, IFN-γ-producing cells were detectable in the blood of both tuberculoid and lepromatous patients at equal levels when exposed to total extracts of M. tuberculosis (Fig. 2B; T-Lep, 22.8 ± 4.6 IFN-γ-producing cells/n = 19; L-Lep, 27.0 ± 5.8 (n = 20); p = 0.70, not significant). These data are consistent with studies indicating that lepromatous patients are specifically unresponsive to protein Ags of M. leprae, yet exhibit functional T cell responses to M. tuberculosis protein Ags (29–31).

Similar to the total extract of M. leprae, the frequencies of IFN-γ-producing cells from patient groups to the lipid extract of M. leprae were strikingly distinct (Fig. 2C), with a mean difference (lipid extract minus medium) in the number of IFN-γ-producing cells equal to 20.2 ± 4.0 (mean ± SEM; n = 19) for tuberculoid patients and 3.0 ± 0.8 (n = 22) for lepromatous patients (p < 0.001). Surprisingly, the frequencies of IFN-γ-producing cells in response to lipid extracts of M. tuberculosis were also greater for tuberculoid (10.0 ± 2.6; n = 31) patients compared with lepromatous patients (3.0 ± 1.0; n = 25; p < 0.05). The data indicate that in contrast to peptide-reactive T cells from lepromatous patients, which are selectively unresponsive to M. leprae Ags, T cells from lepromatous patients exhibit a reduced responsiveness to lipid Ags from multiple mycobacterial species relative to T cells from tuberculoid patients.

We made several other observations regarding the responses of leprosy patients to lipid extracts of mycobacteria. First, a subset of tuberculoid patients did not respond well to the total extract. Proliferation assays ([³H]thymidine incorporation) showed that the T cells did, in fact, respond, suggesting that although responding T cells were present, the ELISPOT did not detect all IFN-γ-producing cells. Second, the data points in Fig. 2, C and D, are not all from the same T-Lep patients. The top four responders to M. leprae (Fig. 2C) and M. tuberculosis (Fig. 2D) lipid extracts represent seven different donors. The data indicate that the most vigorous responders to M. leprae are not necessarily the strongest responders to M. tuberculosis, demonstrating that the data are not the result of four abberant donors. Third, lipid extracts from multiple M. tuberculosis strains were examined. In some cases the frequencies of responding cells were different, which may be explained by a difference in glycolipid composition in a laboratory strain vs a clinical isolate (14). These data are included in Fig. 2D. Finally, a limited number of T-Lep patients were tested with mycobacterial
lipid extracts on multiple occasions. These donors repeatedly responded to the lipid extracts, confirming the finding that tuberculoid patients exhibit higher T cell responses to lipid Ags than lepromatous patients.

Monocyte-derived dendritic cell functions of patients with disseminated leprosy infection are intact

Three possible explanations for the reduced responsiveness to lipid Ags of T cells in lepromatous patients were considered. First, the Ag-presenting function of CD1$^+$ dendritic cells may be reduced, resulting in an inability to prime CD1-restricted T cells. Secondly, T cells from lepromatous patients may exhibit distinct functions, e.g., Th2 cytokine patterns or suppressor functions. Third, the T cells of lepromatous patients may be unresponsive to mycobacterial lipids due to the conserved nature of lipid Ags relative to protein Ags. To evaluate the Ag-presenting function of dendritic cells in lepromatous leprosy, we generated monocyte-derived dendritic cells from healthy donors and leprosy patients. Monocyte-derived dendritic cells from leprosy patients expressed slightly lower CD1 levels than those of healthy donors, but the levels of CD1 expression were comparable across the leprosy spectrum (Fig. 3A), consistent with our earlier report (13). The Ag-presenting function of CD1$^+$ dendritic cells was evaluated using a CD1b-restricted T cell line, LCD4.6 (6). The dendritic cells derived from leprosy patients presented CD1 lipid Ag at the same level as healthy donors across a broad range of Ag (Fig. 3B) and APC concentrations (data not shown). The data indicate that monocytes of lepromatous patients have the capacity to differentiate into CD1 Ag-presenting dendritic cells in vitro.

T cells from lepromatous leprosy patients do not produce Th2 cytokines in response to CD1 lipid Ags

One mechanism of T cell unresponsiveness in leprosy is through the action of CD8$^+$ T suppressor cells (32), which produce IL-4 and thereby inhibit Th1 responses (33). Thus, we considered a role for IL-4-producing CD8$^+$ T cells in preventing CD1-restricted T cell responses. T cells from lepromatous leprosy patients were stimulated with autologous dendritic cells in the presence of M. leprae Ag, and an ELISPOT assay was performed to evaluate the
frequency of IL-4-producing cells. The frequency of IL-4-producing T cells from a lepromatous leprosy patient did not increase dramatically in response to *M. leprae* lipid extract, but did produce IL-4 in response to a polyclonal stimulus, PHA (Fig. 4A, one of five independent donors is shown).

To determine whether CD8 T cells suppressed CD1-restricted T cell responses through some other mechanism, we depleted CD8 T cells before adding dendritic cells and Ag. Depletion of CD8 T cells did not result in an increase in T cell responses to *M. leprae* lipid extracts (Fig. 4B, one of three independent donors is shown), indicating that CD8 T cells do not suppress the CD1-restricted T cell response in lepromatous patients in vitro.

Suppression of Th1 responses can also occur through IL-10 production by T cells or monocytes (34). We therefore investigated the possibility that lepromatous patients’ lack of CD1-restricted T cell responses was due to the production of IL-10. *M. leprae* lipids did not stimulate significant levels of IL-10, in contrast to a polyclonal stimulus (Fig. 4C, one of four independent donors is shown), suggesting that the low levels of IFN-γ in lepromatous patients in response to lipid Ags are not mediated by IL-10 production. Together, the data in Fig. 4 indicate that T cells do not produce Th2 cytokines in response to CD1 lipid Ags, and the CD1-restricted T cells are not subject to suppression by CD8 T cells.

**FIGURE 4.** T cells from lepromatous leprosy patients do not produce Th2 cytokines in response to CD1 lipid Ags. A, IL-4-producing T cells recognizing bacterial extracts were evaluated by ELISPOT as described in Fig. 1 for IFN-γ-producing cells. One representative experiment of five independent donors is shown. Values expressed are the means of triplicate cultures. B, Depletion of CD8 T cells does not enhance the frequency of IFN-γ-producing cells against lipid extracts of mycobacteria. IFN-γ-producing cells were evaluated by ELISPOT. □, Level of IFN-γ-producing cells after CD8 cells were depleted by immunomagnetic selection. One representative experiment of three independent donors is shown. Values expressed are the means of triplicate cultures. C, IL-10-producing cells were evaluated by ELISPOT. One representative experiment of four independent donors is shown. Values expressed are the mean ± SEM of triplicate cultures.

**CD1-restricted T cell repertoire lacks species specific Ag recognition**

T cell recognition of peptide Ags presented by MHC class II is highly specific, discriminating between single amino acid changes within a peptide epitope. MHC class II-restricted T cells derived from leprosy lesions exhibit this high degree of specificity for *M. leprae* peptide epitopes even in comparison with the closely related pathogen *M. tuberculosis* (21, 35). In contrast, there is little evidence indicating species-specific recognition of microbial Ags by CD1-restricted T cells; instead, most clones recognize multiple mycobacterial species (3, 27, 36). We therefore considered the possibility that CD1-restricted T cell responses were primarily cross-reactive. Several CD1a-, CD1b-, and CD1c-restricted T cell clones derived from tuberculoid patients and healthy donors were evaluated for Ag responsiveness to lipid extracts of *M. leprae* and *M. tuberculosis*. We found the majority of CD1-restricted T cell clones (92%) to be cross-reactive with lipid Ags from both *M. leprae* and *M. tuberculosis* (Fig. 5A), although some clones showed stronger responses to *M. tuberculosis* extracts, perhaps due to enrichment of a particular lipid Ag in the *M. tuberculosis* extract. To examine the extent of cross-reactivity of CD1-restricted T cells, we examined a broader range of mycobacteria. A CD1b-restricted T cell line that recognizes mycobacterial lipoarabinomannan (2) responded to extracts from at least four different mycobacterial species, but not extracts from bacteria that do not produce lipoarabinomannan (Fig. 5B).

To quantitate the level of species specificity of MHC-restricted T cells derived from leprosy lesions, we evaluated Ag responsiveness against mycobacterial extracts. CD4+ T cell clones derived from three donors were tested with *M. leprae* or *M. tuberculosis* Ag (total extracts) using MHC class II-matched APCs. T cell clones from tuberculoid lesions tested with MHC class II-matched APCs segregated into four categories (Fig. 5C). The largest group of T cells (50% of total clones or 66% of *M. leprae*-reactive T cells) showed an *M. leprae*-specific Ag response (lower right quadrant). A second group (23% of total, 34% of *M. leprae*-reactive; upper right quadrant) exhibited cross-reactivity between *M. leprae* and *M. tuberculosis* Ags. A third group of T cells (8%) showed *M. tuberculosis*-specific reactivity; presumably these peptide epitopes were not processed sufficiently from *M. leprae* extracts. A fourth group exhibited no reactivity to either *M. leprae* or *M. tuberculosis* Ags. In contrast, T cell clones derived from lepromatous lesions using *M. leprae* Ags lacked Ag-reactive T cells to either *M. leprae* or *M. tuberculosis* Ags (Fig. 5D). To confirm the lack of *M. leprae* reactivity of T cells from lepromatous leprosy, we derived T cells from the blood of lepromatous patients with purified protein derivative from *M. tuberculosis*. T cells derived from the blood of lepromatous patients against *M. tuberculosis* showed no cross-reactivity against *M. leprae* extracts (Fig. 5D). These findings confirm our earlier studies and those of other investigators indicating that lepromatous leprosy patients have little or no *M. leprae*-specific MHC class II responses (21, 29–31). If one compares that data shown in Fig. 5, A and C, it is apparent that although MHC class II-restricted T cells are both species specific and cross-reactive, CD1-restricted T cells are predominantly cross-reactive.

**Discussion**

The striking finding of the present study was that the frequencies of both *M. leprae* and *M. tuberculosis* lipid-reactive T cells were reduced in lepromatous patients compared with tuberculoid patients. This was in contrast to peptide-reactive T cells of lepromatous patients, where frequencies were reduced against *M. leprae*.
shown that the number of CD1-derived from lepromatous patients in vitro, we have previously although we found that monocyte-derived dendritic cells can be T cells in lepromatous leprosy is an inability to present CD1 Ags; a potential mechanism for the reduced frequency of CD1-restricted (37–39) where CD1-restricted T cells are selected (40). A second restricted T cells in lepromatous leprosy are deleted in the thymus (32, 33); however, we cannot exclude the possibility that CD1-elimination of cross-reactive T cells, most likely in the periphery to cover a broader spectrum of microbial epitopes. Whereas CD1 restrictive T cell repertoire, the CD1-restricted T cell repertoire is limited to recognition of cross-reactive Ags, imparting a distinct role in the host response to immunologically related pathogens.

There are a number of possible explanations for the reduction in the mycobacteria-reactive, CD1-restricted T cell repertoire in lepromatous leprosy. We speculate that the mechanism is through elimination of cross-reactive T cells, most likely in the periphery (32, 33); however, we cannot exclude the possibility that CD1-restricted T cells in lepromatous leprosy are deleted in the thymus (37–39) where CD1-restricted T cells are selected (40). A second potential mechanism for the reduced frequency of CD1-restricted T cells in lepromatous leprosy is an inability to present CD1 Ags; although we found that monocyte-derived dendritic cells can be derived from lepromatous patients in vitro, we have previously shown that the number of CD1+ dendritic cells in lepromatous lesions are reduced (13). These two mechanisms are not mutually exclusive and, in fact, may function together to prevent generation of CD1-restricted T cell responses in lepromatous leprosy. We considered a third possibility, the existence of an altered CD1-restricted T cell repertoire in lepromatous patients. However, this was deemed unlikely in light of our findings that, in response to lipid Ags, T cells from lepromatous leprosy patients did not produce the Th2 cytokines characteristic of lepromatous leprosy (24, 33). A fourth possibility is that the CD1-restricted T cell repertoire is mobilized in tuberculoid patients by exposure to Ag and not in unresponsive lepromatous patients. Although the frequency of CD1-restricted T cells may increase upon exposure to microbial Ags (27, 41), we favor the interpretation that T cells from lepromatous patients are unresponsive to lipid Ags of multiple mycobacterial species because 1) T cells of lepromatous patients did not respond to lipid extracts of the closely related M. tuberculosis (the present study); 2) CD1-restricted T cells are elicited from nonimmunized donors (2, 26, 42); and 3) CD1-restricted T cell lines from lepromatous patients have not been derived (our unpublished observations).

To identify a mechanism for the decrease in lipid-reactive T cells of lepromatous patients, we examined the species specificity of T cell clones derived from tuberculoid patients. We found that the majority of M. leprae-reactive CD1-restricted T cell clones (92%) were cross-reactive for multiple mycobacterial species. In contrast, the repertoire of M. leprae-reactive MHC-restricted T cells was predominantly species specific (66%), with a limited number of T cell clones cross-reactive (34%) with M. tuberculosis. One prediction arising from our data indicating that CD1-restricted T cells recognize conserved microbial Ags is cross-protection (43, 44) against other mycobacterial infections. Studies have demonstrated that vaccination with the attenuated mycobacterial strain bacillus Calmette-Guérin confers protection against leprosy infection (45). Conversely, a negative consequence of cross-reactive T cell recognition is that it predisposes toward self-recognition and autoimmunity (46, 47) or elimination through negative selection. Therefore, one might predict increased susceptibility to infection by other mycobacterial species in lepromatous patients in whom CD1-restricted T cells are reduced. Patients with lepromatous leprosy, in fact, have increased susceptibility to tuberculosis infection compared with tuberculoid patients (48). Immune protection against mycobacterial infection may thus require the complementary Ag recognition properties of peptide- and lipid-reactive T cells to cover a broader spectrum of microbial epitopes. Whereas CD1 and MHC bind and present distinct Ag structures to T cells, the
functions of MHC- and CD1-restricted T cells against mycobacterial overlap, i.e., production of cytokines for macrophage activation (2, 24, 33) and lysis of infected cells to control growth of the bacteria (6, 49).

We found that although CD1-restricted T cell clones are cross-reactive, recognizing conserved lipid Ags present in multiple mycobacterial species, MHC-restricted T cell clones recognized predominantly species-specific Ags. Peptide Ags are readily altered by mutating the gene from which they are encoded and therefore represent a virtually unlimited number of Ags against which MHC-restricted T cells must be mobilized. In contrast, Ags recognized by CD1-restricted T cells include a conserved set of self (50–52) and microbial Ags (1, 2, 27, 53) that are vital to the structural integrity of the cellular envelope and require multiple enzymes to assemble complex lipids and glycolipids (54). Thus, selection of conserved structures is favored in lipid in contrast to proteins Ags.

Our findings indicating the conserved nature of CD1 Ag recognition may provide insight into the diversity of TCRs on lipid- vs peptide-reactive T cells; they suggest that the TCR repertoire of group 1 CD1-restricted T cells is shaped by the Ags recognized. Diversity in the MHC-restricted TCR repertoire is required to maintain recognition of microbial peptides derived from a virtually unlimited number of microbial proteins. In contrast, CD1-restricted NK T cells express a highly limited set of TCRs (5) that may be intrinsically deficient in $\alpha/\beta$ TCR combinations (56) to recognize a restricted set of Ags (5). Group 1 CD1-restricted T cells appear to occupy a middle ground between these two extremes. CD1a-, CD1b-, and CD1c-restricted T cells express a greater diversity of $\alpha\beta$, $\gamma\delta$, and $\delta\gamma$ segments and junctional diversity of CDR3 regions (57) than CD1d-restricted NK T cells (55, 58) and recognize a growing list of microbial lipid Ags (1, 2, 27, 53, 59). Our initial studies of CD1-restricted T cells derived from the lesions of tuberculoid patients indicated that the T cells produced macrophage-activating cytokines and lysed Ag-pulsed targets (2). We later determined that CD1-restricted T cells could lyse mycobacteria-infected cells through two distinct mechanisms (49), including the release of cytolytic granule proteins, which provides a mechanism by which T cells can directly inhibit mycobacterial growth (6, 64). These findings also raise the possibility that engineering optimal vaccines should include both peptide and lipid Ags in an effort to elicit both MHC and CD1-restricted T cells against infection. Furthermore, the inclusion of lipid Ags in a vaccine against a particular pathogen might engender cross-protection against immunologically related species.

Acknowledgments
We thank Eleanor Cabrera for expert technical assistance and the University of California–Los Angeles Flow Cytometry Core and Immunology Core laboratories for the use of their facilities.

Disclosures
The authors have no financial conflict of interest.

References


