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Evidence for Human CD4^{+} T Cells in the CD1-Restricted Repertoire: Derivation of Mycobacteria-Reactive T Cells from Leprosy Lesions[1]

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Both the CD4⁻CD8⁻ (double negative) and CD4⁻CD8⁺ T cell lineages have been shown to contain T cells which recognize microbial lipid and glycolipid Ags in the context of human CD1 molecules. To determine whether T cells expressing the CD4 coreceptor could recognize Ag in the context of CD1, we derived CD4⁺ T cell lines from the lesions of leprosy patients. We identified three CD4⁺ *Mycobacterium leprae*-reactive, CD1-restricted T cell lines: two CD1b restricted and one CD1c restricted. These T cell lines recognize mycobacterial Ags, one of which has not been previously described for CD1-restricted T cells. The response of CD4⁺ CD1-restricted T cells, unlike MHC class II-restricted T cells, was not inhibited by anti-CD4 mAb, suggesting that the CD4 coreceptor does not impact positive or negative selection of CD1-restricted T cells. The CD4⁺ CD1-restricted T cell lines produced IFN-γ and GM-CSF, the Th1 pattern of cytokines required for cell-mediated immunity against intracellular pathogens, but no detectable IL-4. The existence of CD4⁺ CD1-restricted T cells that produce a Th1 cytokine pattern suggests a contributory role in immunity to mycobacterial infection. *The Journal of Immunology,* 2000, 164: 4790–4796.

Until recently, Ag presentation to T cells was defined by a limited number of proteins encoded within the MHC locus. It is now clear that additional proteins within the MHC locus (1, 2), as well as proteins encoded outside the MHC locus, can also present Ags to T cells. The best studied of these non-MHC-encoded proteins is the CD1 family (3, 4). The pathway of CD1-presented Ags diverges from that of classical MHC processing, indicating that the CD1 Ag presentation may be complementary to the MHC pathways. The most surprising finding of the CD1 Ag-presenting system is that the Ags presented by CD1 are not peptide, but rather lipid or glycolipid in nature (5, 6).

The CD1 proteins have been classified into two subgroups based on sequence similarity: The group 1 proteins which include human CD1a, CD1b, and CD1c molecules are much more closely related to one another than they are to the fourth human protein, CD1d. Group 2 proteins include human CD1d and murine CD1. Most of our knowledge about group 1 CD1-restricted T cells and their role in microbial infection derives from studies investigating the host response to mycobacteria. Immunity to mycobacterial infection requires both MHC class I- and class II-restricted T cells (7, 8) which are directed against peptide Ags. However, mycobacteria are characterized by a complex lipid and glycolipid envelope, which has potent immunostimulatory activity for B cells and monocytes (9, 10). Previous studies from our laboratories have shown that this complex mycobacterial lipid envelope is a rich source of CD1-restricted T cell Ags as well (4, 6, 11). The general structural features of these T cell Ags are a hydrophobic region (fatty acid chains) coupled to a polar or charged hydrophilic end. The large hydrophobic pockets identified in murine CD1 (12) are thought to be capable of accommodating the fatty acid portions of these Ags. Direct binding studies have confirmed that the lipid portion of CD1 ligands bind to CD1b (13).

As an initial step toward defining the in vivo relevance of CD1 Ag presentation, we have studied the involvement of CD1 in the immune response to *Mycobacterium leprae*, the causative agent of leprosy. Infection with this organism provides an opportunity to study the human immune response to a mycobacterial pathogen in situ, since much of the pathology is localized to the skin where the relevant lesions can be easily sampled. Furthermore, leprosy presents as a spectrum in which clinical disease correlates with different levels of responsiveness to *M. leprae*. At one pole of the spectrum are patients with strong cell-mediated immunity to *M. leprae* and a localized form of 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.

Previous studies have shown that only CD4$^-$ (CD8$^+$ and double negative (DN))[4] T cells recognize mycobacterial Ags in the context of CD1 (4, 6, 14, 15). However, CD4$^+$ T cells predominate in the lesions of tuberculoid leprosy (16) and CD4 can be expressed on group 2 CD1-restricted T cells (17). To determine whether T cells expressing the CD4 coreceptor could be selected by group 1 CD1 proteins, we derived CD4$^+$ T cell lines from the lesions of leprosy patients. We identified three CD4$^+$ *M. leprae*-reactive, CD1-restricted T cell lines: two that recognize mycobacterial Ags in the context of CD1b and one that recognizes an as yet unidentified lipid Ag in the context of CD1c. Monoclonal Abs that prevent CD4$^+$ HLA-DR-restricted T cells from responding to Ag had no effect on CD4$^+$ or DN CD1-restricted T cells, indicating that the CD4 coreceptor does not impact positive or negative selection of CD1-restricted T cells. The CD4$^+$, CD1-restricted T cell lines produced IFN-γ and GM-CSF, the Th1 pattern of cytokines required for cell-mediated immunity against intracellular pathogens, but no detectable IL-4. Therefore, the existence of CD4$^+$ CD1-restricted T cells that produce a Th1 cytokine pattern suggests a contributory role in immunity to mycobacterial infection.

Materials and Methods

Patients and clinical specimens

Leprosy patients were recruited on a volunteer basis from the ambulatory population seen at the Dermatology Clinics at the University of Southern California Medical Center and the University of Miami Medical Center. Clinical classification of patients with symptomatic *M. leprae* infection was done according to the criteria of Ridley and Jopling (18). Skin biopsy specimens (6-mm diameter) containing both epidermis and dermis were obtained by standard punch technique following informed consent. Blood samples for isolation of PBMC were obtained by venipuncture from leprosy patients and from healthy volunteer laboratory personnel who served as control subjects and as a source for generating CD1$^+$ APCs. PBMC were isolated using density gradient centrifugation (Ficoll-Paque; Pharmacia, Uppsala, Sweden).

Ags and Abs

Extracts of *M. leprae*, *M. tuberculosis* (strain H37Rv), and *M. phlei* were prepared by probe sonication (19). To prepare non-peptide Ags, sonicated mycobacteria were treated with proteinase K (0.7 mg/ml, Boehringer Mannheim, Indianapolis, IN) for 30 min at 60°C, and the enzyme was heat-inactivated for 10 min at 70°C. Purified mycobacterial lipids and lipoglycans were isolated as described previously (5, 11, 20, 21). The following Abs were used for flow cytometry: IgG controls (Sigma, St. Louis, MO), OKT6 (anti-CD1a (22)), BCD1b3.1 (anti-CD1b (23, 24)), F10/21A3 (anti-CD1c (25)); OKT3 (anti-CD3; American Type Culture Collection (ATCC), Manassas, VA); OKT4 (anti-CD4; ATCC); OKT8 (anti-CD8; ATCC), BMA 031 (anti-TCR$\alpha\beta$; Caltag, Burlingame, CA); 5A6.E9 (anti-TCR$\gamma\delta$; Caltag); HP-3D9 (anti-CD94; PharMingen, San Diego, CA); DX12 (anti-CD161/NKR-P1A; PharMingen).

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

CD1 expression on monocytes was induced in vitro with a combination of recombinant human GM-CSF (200 U/ml) and recombinant human IL-4 (100 U/ml) for 72 h as described elsewhere (4, 4, 26, 27). Cells were harvested using incubation in PBS/0.5 mM EDTA to detach adherent cells and analyzed for CD1 expression by flow cytometry (4) or irradiated (5000 rad) and used as APCs.

T cell lines and proliferation assays

T cell lines were derived from leprosy lesions and the blood of healthy donors as described previously (6, 28), although CD4 and CD8 T cells were not depleted. Briefly, cells were extracted from lesions with a tissue sieve and lymphocytes were isolated by density gradient centrifugation. HLA-DR-restricted T cell lines were initiated in the presence of irradiated autologous PBMC and mycobacterial extract, whereas CD1-restricted T
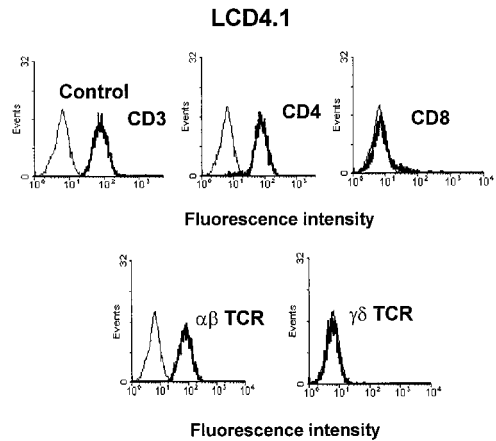
[4] Abbreviations used in this paper: DN, double negative; LAM, lipoarabinomannan; GMM, glucose monomycolate; PIM, phosphatidyl inositol mannoside.

FIGURE 1. CD4$^+$ T cell lines derived from tuberculoid leprosy lesions. Flow cytometric analysis of T cell line LCD4.1 demonstrating CD3, CD4, and TCR$\alpha\beta$ expression. Lymphocytes were extracted from skin biopsies, cultured with autologous (first two stimulations) and heterologous (all other stimulations) CD1$^+$ APCs and *M. leprae*. T cell lines LCD4.2 and LCD4.3 showed the same CD4$^+$TCR$\alpha\beta^+$ phenotype. Results shown are representative of three or more analyses.

cells were cultured in the presence of irradiated autologous CD1$^+$ APCs and extract (4). T cell lines were maintained by serial antigenic stimulation in recombinant IL-2 (1 nM; Chiron Diagnostics, Norwood, MA) supplemented medium. For measurement of Ag-specific proliferation, T cells (1 × 10^4) were cultured with varying numbers (usually 1 × 10^4) of irradiated (5000 rad) heterologous CD1$^+$ APCs in culture medium (0.2 ml) in the presence or absence of bacterial Ags for 3 days in microtiter wells (in triplicate) at 37°C in a 7% CO$_2$ incubator. Cells were pulsed with [^{3}H]thymidine (1 μCi/well; ICN Biomedicals, Costa Mesa, CA) 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. Alternatively, THP-1 cells expressing distinct isoforms of CD1 were used as APCs (29). To determine the role of CD4 accessory interactions, T cells were cultured with a neutralizing CD4 Ab (clone QS4120; Calbiochem, San Diego, CA) or isotype-matched control Ab for 30 min, cells were washed to remove unbound Ab, and T cells were added to APCs and Ag. Cytokine release from T cells was measured by ELISA after stimulation with CD1-positive APCs and Ag or media for 24 h. ELISAs (IFN-γ and IL-4; Endogen, Woburn, MA; GM-CSF, PharMingen, San Diego, CA) were performed according to the instructions of the manufacturers.

Results

CD4$^+$ CD1-restricted T cells in leprosy lesions

We derived T cell lines from the granulomatous lesions of patients with tuberculoid leprosy in the presence of CD1$^+$ APCs and an extract of *M. leprae*. Flow cytometry and T cell proliferative assays revealed that three cell lines (LCD4.1 (Fig. 1), LCD4.2, and LCD4.3) from three different patients expressed CD4 and TCR$\alpha\beta$ and the ability to respond to *M. leprae* Ag in the presence of heterologous CD1$^+$ APCs. These three cell lines were therefore selected for further study.

CD4$^+$ T cells derived from tuberculoid leprosy lesions have been shown to produce a Th1 cytokine pattern (30, 31). These T cell lines were peptide-reactive HLA-DR-restricted and have generally been thought to contribute to immunity against *M. leprae*. To determine the cytokine production from CD4$^+$ T cells derived by culture with CD1$^+$ APCs, we stimulated the cell lines with CD1$^+$ APCs and *M. leprae*. The T cell lines produced IFN-γ, a potent stimulus for MHC class II expression, but no detectable IL-4 (Fig. 2). We have previously shown that both GM-CSF and CD1 are elevated in tuberculoid as compared with lepromatous lesions (32, 33). CD1 expression in vitro is dependent on cytokines, most notably GM-CSF (4, 26). The CD4$^+$ T cell lines also
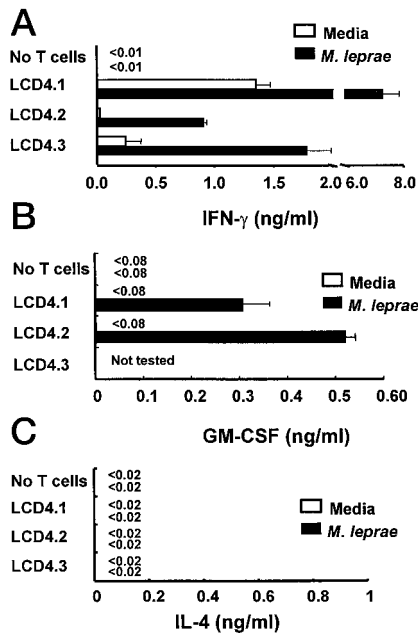
FIGURE 2. Cytokine production from CD4$^+$ T cell lines derived from leprosy skin lesions. CD4$^+$ T cells (1 × 10^4) were cultured in the presence of heterologous CD1$^+$ APCs (1 × 10^4) in the presence or absence of sonicated *M. leprae* (1:1000 dilution of extract). Supernatants were harvested after 24 h and IFN-γ (*A*), GM-CSF (*B*), and IL-4 (*C*) were measured by ELISA. Values (means ± SEM) shown are representative of three or more independent experiments for IFN-γ and IL-4 and at least two independent experiments for GM-CSF.

FIGURE 3. CD1c-restricted CD4$^+$ T cell line LCD4.1. *A*, T cells were cultured as described in Fig. 2 and pulsed with [^{3}H]thymidine (4 h) after 72 h of culture. Cells were harvested, and ^{3}H incorporation was measured by a scintillation counter. *B*, T cell supernatants (24 h) prepared as in Fig. 2 were measured for IFN-γ production by ELISA. *C*, T cells (2 × 10^4) were cultured with THP-1 cells (2 × 10^4) transfected with distinct CD1 isoforms in the presence or absence of *M. leprae* (1:1000 dilution of extract). Supernatants were harvested at 24 h and IFN-γ was measured by ELISA. Values (means ± SEM) shown are representative of at least three independent experiments.

produced GM-CSF in response to Ag. Together, these data suggest that CD4 T cell lines stimulated under conditions to activate CD1-restricted T cells produce cytokines that would enhance Ag presentation through both the MHC class II and CD1 pathways.

To determine whether the T cell lines recognized *M. leprae* in the context of CD1, we used neutralizing Abs to the three isoforms of CD1 present on dendritic cells in leprosy lesions, CD1a, CD1b, and CD1c (33). Fig. 3, *A* and *B*, shows that Abs to CD1c blocked the proliferation and IFN-γ production of the CD4$^+$ T cell line LCD4.1, indicating that LCD4.1 recognized *M. leprae* Ag in the context of CD1c. The CD1c restriction of LCD4.1 was confirmed using the monocytic cell line, THP-1, transfected with distinct CD1 isoforms. CD1c-expressing THP-1 cells induced IFN-γ production from LCD4.1 in the presence of *M. leprae* (Fig. 3*C*), whereas CD1b-transfected THP-1 did not induce IFN-γ production. Similarly, LCD4.1 was activated to produce IFN-γ in the presence of *M. leprae*-pulsed CD1c, but not CD1b-transfected HeLa cells (data not shown).

In contrast to LCD4.1, which was CD1c restricted, Abs to CD1b inhibited the proliferation and IFN-γ production of LCD4.2, indicating that CD1b presented *M. leprae* Ag to LCD4.2 (Fig. 4, *A* and *B*). Interestingly, THP-1 cells transfected with CD1a, CD1b, or CD1c did not induce IFN-γ production from LCD4.2 (data not shown). The third CD4$^+$ T cell line, LCD4.3, produced IFN-γ, which was inhibited by neutralizing Ab to CD1b, but not by anti-CD1a or anti-CD1c (Fig. 4*C*), indicating CD1b restriction. Finally, to demonstrate that this population of T cells was present in the pool of peripheral blood T cells, we also derived CD1-restricted CD4$^+$ T cells from the blood of a healthy donor (BCD4.1, Fig. 4*D*). We conclude therefore that CD4$^+$ T cells belong to the repertoire of T cells that recognize microbial Ags in the context of group 1 CD1 proteins.
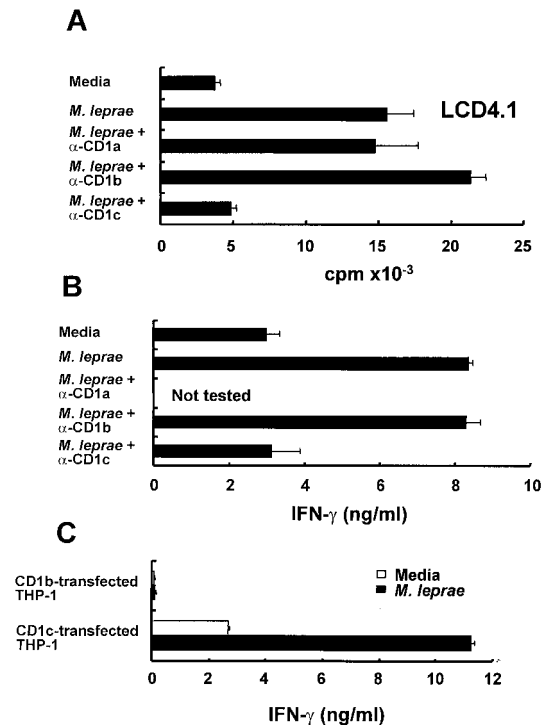
Expression of NK receptors on CD4$^+$ CD1-restricted T cells derived from leprosy lesions

Murine CD1-restricted T cells are characterized by their expression of NK receptors, in particular NK1.1 (34), and therefore are often referred to as NK T cells. Activation of NK T cells through NK1.1 results in robust IFN-γ production (35) in contrast to the large amounts of IL-4 produced after TCR stimulation (36, 37), indicating distinct effector functions for NK T cells depending on which receptor is stimulated. CD94 is a human NK receptor (38) that has also been identified on CD1d-restricted T cells (39). We evaluated the expression of these NK receptors on CD1-restricted CD4$^+$ T cell lines derived from leprosy lesions. We found that NKR-P1A was negative on both LCD4.1 and LCD4.3, whereas CD94 expression was variable; LCD4.1 was CD94 negative and LCD4.3 was CD94 positive (Fig. 5). Additional cell lines must be evaluated to gain a better understanding of the expression of NK receptors on group 1 CD1-restricted T cells, but the lack of NKR-P1A would appear to distinguish these T cells from NK T cells (34, 39).

CD4$^+$ CD1-restricted T cells recognize non-peptide Ags

To date, human CD1-restricted T cells have been shown to recognize lipid and glycolipid Ags (5, 6, 11, 19), thus we investigated the nature of the Ags that activated CD4$^+$ CD1-restricted T cells. LCD4.1 T cells were activated by extracts from *M. leprae*, *M. tuberculosis,* and *M. phlei*, (Fig. 6*A*), suggesting that the Ag was

FIGURE 4. CD1b-restricted CD4$^+$ T cell lines LCD4.2 and LCD4.3. *A*, T cells were cultured as described in Fig. 2 and pulsed with [^{3}H]thymidine (4 h) after 72 h of culture. Cells were harvested, and ^{3}H incorporation was measured by a scintillation counter. *B*, T cell supernatants prepared as in Fig. 2 were measured for IFN-γ production by ELISA. *C*, IFN-γ production by CD4$^+$ T cell line LCD4.3. *D*, CD4$^+$ CD1b-restricted T cell line, BCD4.1, derived from the peripheral blood of a healthy donor. Values (means ± SEM) shown are representative of three or more independent experiments.
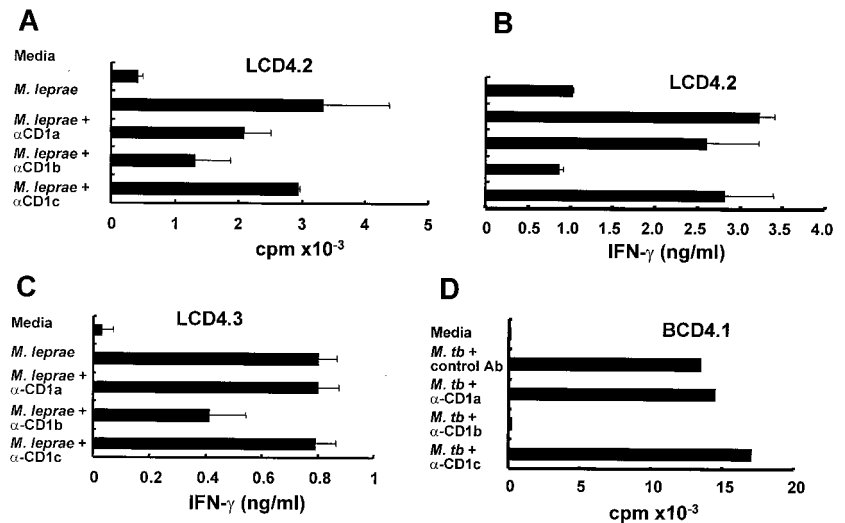
structurally conserved in different mycobacterial species. To determine whether the Ag was a protein, *M. tuberculosis* extract was treated with proteinase K. Fig. 6*B* demonstrates that proteinase K treatment of *M. tuberculosis* had no effect on the ability to stimulate LCD4.1, whereas proteinase K abrogated the ability of *M. tuberculosis* to activate an HLA-DR-restricted T cell line LDR-2 (Fig. 6*C*), indicating that LCD4.1 recognizes a non-peptide Ag. To determine whether the Ags previously shown to stimulate CD1b- and CD1c-restricted T cells could activate LCD4.1, lipoarabinomannan (LAM), glucose monomycolate (GMM), and mycolic acids were added to T cell cultures. None of these three CD1 ligands activated LCD4.1 (Fig. 6*D*). Our data indicate that LCD4.1 recognizes a previously unidentified non-peptide mycobacterial Ag in the context of human CD1c.

CD1b and CD1c ligands were also tested on the CD4$^+$ CD1b-restricted T cell line LCD4.2 (Fig. 7*A*). LAM and phosphatidyl inositol mannoside (PIM) induced IFN-γ production from LCD4.2, whereas GMM and mycolic acids did not. We have previously identified two CD1b-restricted CD4$^-$CD8$^-$ T cell lines that recognize PIM and LAM (6, 40). To confirm that LCD4.2

recognized glycolipid Ag in the context of CD1b, we used neutralizing Abs to CD1 molecules (Fig. 7*B*). Ab to CD1b inhibited IFN-γ production from LCD4.2 by 82%, whereas anti-CD1c inhibition was weak by comparison. As indicated above, THP-1 cells transfected with CD1b did not stimulate LCD4.2, probably due to the requirement for the mannose receptor for uptake of LAM and PIM for CD1b Ag presentation (41). Taken together, with the data from Fig. 4, we conclude that T cell line LCD4.2 recognizes PIM in the context of CD1b.

To determine the Ag responsiveness of the CD1b-restricted T cell line LCD4.3, we also tested the known mycobacterial CD1 Ags. LCD4.3 responded to *M. leprae* and mycolic acid (Fig. 7*C*), a previously recognized CD1 Ag (5), but exhibited no response to two other CD1b Ags, LAM (6) and GMM (11). The data indicate that all three CD1-restricted CD4$^+$ T cell lines derived from leprosy lesions recognized non-peptide Ags.

Neutralization of CD4 on the cell surface does not inhibit CD4$^+$ CD1-restricted T cells

The CD4 protein plays an important role in the interaction of HLA-DR-restricted CD4$^+$ T cells and their cognate APCs both in the thymus and the periphery (42–44). We examined whether neutralizing Abs for CD4 influence the response of CD4$^+$ CD1-restricted T cells to mycobacterial Ag. CD4 Ab had minimal effect on the response of LCD4.1 or LCD4.3 to *M. leprae* (Fig. 8, *A* and *B*), whereas the Ab inhibited the proliferation of an HLA-DR-restricted T cell line, D103-5 (45, 46), by 83% (Fig. 8*C*). These data indicate that the CD4 accessory interaction between the T cell and APC is not required for the response of CD4$^+$ CD1-restricted T cells.

Discussion

The established paradigm for Ag presentation has been one of MHC-encoded molecules presenting peptide Ags to T cells. The paradigm holds that CD8$^+$ T cells recognize 8–10 aa peptides presented by MHC class I molecules and CD4$^+$ T cells recognize 13–17 aa peptides presented by MHC class II molecules. Recent studies involving the non-MHC-encoded family of CD1 proteins challenges this view and indicates that T cells recognize lipid Ags in the context of CD1 Ag-presenting molecules. The first human CD1-restricted T cells described were CD4$^-$CD8$^-$ DN expressing either αβ or γδ TCRs (47). DN T cells represent a minor subset of
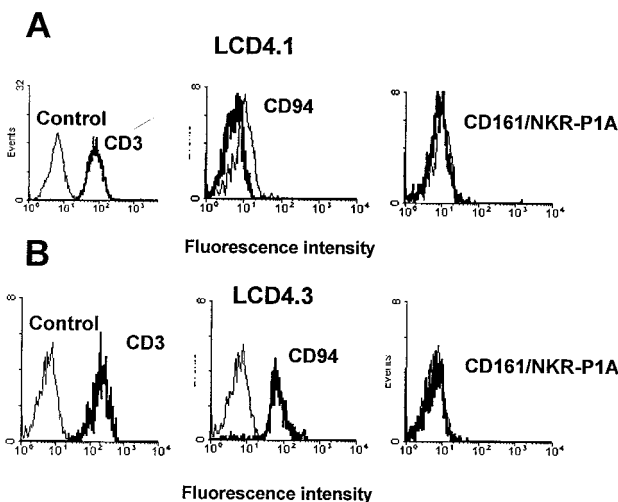
FIGURE 5. Flow cytometric analysis of NK receptor expression on CD4$^+$ CD1-restricted T cells derived from leprosy lesions. T cell lines LCD4.1 (*A*) and LCD4.3 (*B*) were stained with mAbs specific for NK receptors CD94 and CD161 (NKR-P1A). The histograms on the *left* represent the TCR-associated protein CD3.

FIGURE 6. CD1c-restricted T cell line LCD4.1 recognizes a common mycobacterial non-peptide Ag not previously shown to activate CD1-restricted T cells. *A*, T cells were cultured as described in Fig. 2 in the presence of three mycobacterial extracts, and IFN-γ production was measured by ELISA. *B*, T cells were cultured with APCs and untreated or proteinase K-treated *M. tuberculosis* extract, and proliferation was measured by [^{3}H]thymidine incorporation. *C*, T cells from the HLA-DR-restricted T cell line LDR2 were cultured as described in *B* except that the APCs used were from a HLA-DR-matched donor. Proliferation was measured by [^{3}H]thymidine incorporation. *D*, T cells were cultured with previously characterized non-peptide CD1 ligands, LAM, GMM, and mycolic acid, and proliferation was measured by [^{3}H]thymidine incorporation. Values (means ± SEM) expressed are representative of two or more independent experiments.
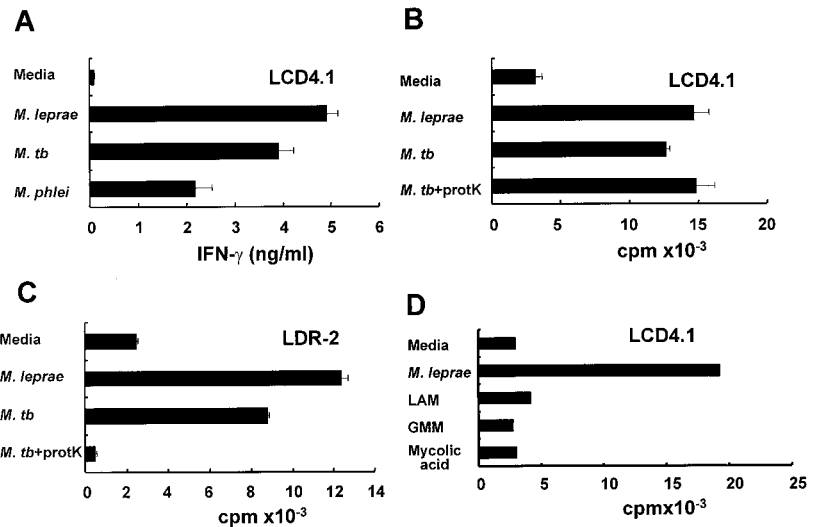
the T cell repertoire, suggesting CD1-restricted T cells might play a minor role in immune responses. However, recent studies indicate that human CD1-restricted T cells can also express CD8αβ heterodimers (14, 15). The present study provides evidence that T cells expressing CD4 and TCRαβ may recognize foreign Ags in the context of CD1 molecules. Three CD4$^+$ T cell lines were derived from leprosy lesions that recognized non-peptide Ags in the context of CD1b or CD1c. These T cell lines produced IFN-γ and GM-CSF, but no detectable IL-4, and the response of the T cells

did not require CD4 accessory interaction, in contrast to a CD4$^+$ HLA-DR-restricted T cell line. The existence of human CD4$^+$TCRαβ$^+$ T cells that are CD1 restricted suggests that the T cell response to Ag presented by CD1 encompasses the complete repertoire of T cells.

One unresolved question regarding CD1-restricted T cells is their representation in different subsets of the T cell repertoire. Our earlier studies demonstrated that CD1-restricted T cells can express CD8 or be CD4 and CD8 negative (DN) (4, 6, 14, 47). The
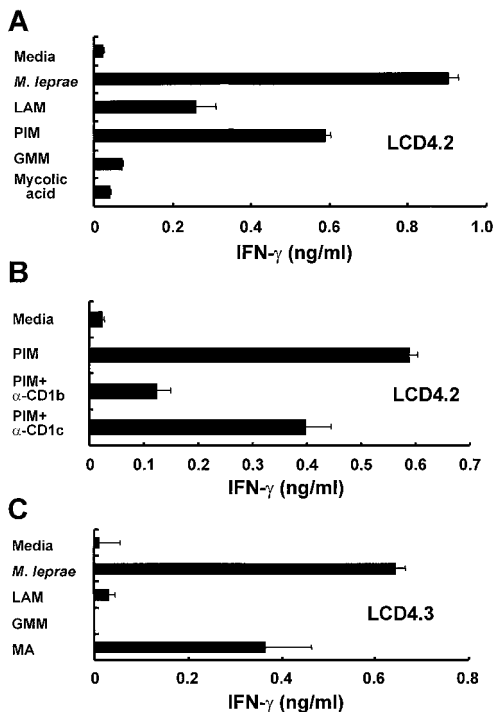

FIGURE 7. CD1b-restricted T cell lines LCD4.2 and LCD4.3 recognize previously identified CD1 ligands. *A*, LCD4.2 responds to PIM. T cells were cultured as described in Fig. 2 in the presence of LAM, PIM, GMM, and mycolic acid, and IFN-γ production was measured by ELISA. *B*, T cells were cultured as above in the presence of neutralizing CD1b or CD1c Abs. IFN-γ production was measured by ELISA. *C*, LCD4.3 responds to mycolic acid. T cells were cultured as above and IFN-γ production was measured by ELISA. Values (means ± SEM) shown are representative of two or more independent experiments.
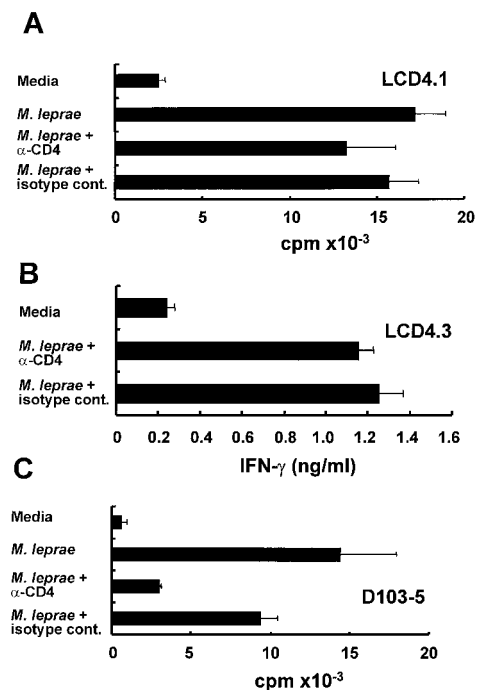
FIGURE 8. MHC class II interaction with CD4 is not required for CD1-restricted T cell activation. T cells were cultured as described in Fig. 2 in the presence of neutralizing CD4 or isotype control Abs. In *C*, the APCs were from a HLA-DR-matched donor. Proliferation (*A* and *C*) or IFN-γ production (*B*) was used to measure T cell activation. Values (means ± SEM) expressed are representative of three or more independent experiments.

present study now indicates that CD1-restricted T cells can express $CD4^+$ coreceptors. Furthermore, both $CD4^+$ and $CD4^-$ T cells have the capacity to recognize the same ligand (PIM) in the context of CD1b (6). In this study, we demonstrated that CD4 accessory interaction is not required for T cell recognition, thus one might conclude that CD4 has no contributory role to the ability of CD1-restricted T cells to recognize Ag or an inhibitory role in preventing such recognition. These findings, however, do not preclude that membrane reorganization and signaling of CD4 could occur in a normal fashion (48–50).

Our data may provide some insight into the role of coreceptors in selection of CD1-restricted T cells. Most of our understanding of selection of CD1-restricted T cells derives from studies in mice. CD1-restricted T cell selection does not require MHC class II molecules (17, 51) and although a role for MHC class I has not been ruled out, the ligand responsible is most likely CD1 itself (52). Since CD1-restricted T cells 1) can be $CD4^+$ or DN (34), 2) can recognize the same glycolipid Ag regardless of CD4 expression (6), and 3) do not require CD4 accessory interactions, our findings indicate that MHC class II is not required for selection of human CD1-restricted T cells and that CD4 is not required for positive selection. The existence of $CD4^+$ CD1-restricted T cells further suggests that CD4 does not negatively select against CD1 responses.

Using leprosy as a model of intracellular infection to evaluate the role of CD1 in human immune responses, we have derived $CD4^+$ CD1-restricted T cells from lesions of patients with tuberculoid leprosy, those patients able to localize the infection and mount T cell responses against the pathogen. $CD4^+$ T cells predominate in the lesions of tuberculoid patients (16) and $CD4^+$ T cell clones derived from these lesions produce cytokines that promote cell-mediated immune responses (30). We have also found a strong correlation between the expression of three forms of CD1 (i.e., CD1a, CD1b, and CD1c) known to be involved in the presentation of mycobacterial lipid Ags and the presence of effective host immunity to *M. leprae* (33). CD1 expression in tuberculoid lesions was shown to be primarily restricted to a mature dendritic cell population, and in vitro functional studies revealed that dendritic cells bearing the phenotype found in tuberculoid lesions were strikingly efficient at presenting mycobacterial lipid Ags to CD1b-restricted T cells. Since all three group 1 CD1 proteins can present lipid Ags to T cells (5, 14, 19), these results support the hypothesis that lipid Ag presentation by the CD1 system plays a beneficial role in host immunity to microbial pathogens in vivo and that $CD4^+$ CD1-restricted T cells participate in the local immune response.

Upon activation by mycobacterial Ag, $CD4^+$ CD1-restricted T cells produced IFN-γ and GM-CSF similar to DN and $CD8^+$ T cells. IFN-γ is a critical cytokine for immunity to mycobacterial infection as evidenced by the greater susceptibility to infection in IFN-γ knockout mice and the greater IFN-γ expression seen in tuberculoid leprosy (32, 53, 54). GM-CSF induces CD1 on monocytes, which would lead to a greater frequency of Ag presentation to CD1-restricted T cells. Together, our data suggest that $CD1^+$ dendritic cells at the site of disease present lipid Ags to $CD4^+$ T cells, leading to the Th1 cytokine response and increased CD1 expression. In this manner, $CD4^+$ CD1-restricted T cells can contribute to cell-mediated immune responses for host defense against intracellular pathogens. In summary, our findings extend the universe of CD1-restricted T cell repertoire to include $CD4^+$ TCR$\alpha\beta$ T cells and identifies these cells as part of the immune response that combats infection.

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