

Immunodeficiency and other clinical immunology

CD1-restricted T-cells influence IgG subclass and IgE production

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Background: Human CD1 has recently emerged as a third family of antigen-presenting molecules that is distinct from either major histocompatibility complex class I or class II. **Objective:** We investigated whether the CD1b-restricted T-cell interaction with antigen alters human IgG subclass and IgE isotype production.

Methods: CD1b-restricted antigen-specific T cells derived from the skin lesion of a patient with leprosy were stimulated with their cognate antigen, lipoarabinomannan (LAM) of *Mycobacterium leprae*, in the presence of CD1⁺ antigen-presenting cells and tested for their ability to alter IgG subclass and IgE production from IgD⁺ B cells.

Results: CD1-restricted T cells cultured with CD1⁺ antigen-presenting cells in the absence of LAM induced IgG1, IgG3, IgG4, and IgE, whereas CD1b-restricted T cells cultured in the presence of LAM induced IgG1 and IgG3 and inhibited production of IgG4 and IgE. Production of IgG4 and IgE was rescued in the CD1-restricted system by the addition of anti-interferon- γ . IgG2 production was not induced under any circumstances.

Conclusion: In this study we demonstrated that a specific CD1b-restricted T-cell line can behave similarly to classically-restricted T_H1-type T cells. CD1b-restricted T-cells of this type may regulate immune responses to microbial pathogens by simultaneously enhancing cell-mediated immunity and downregulating IgG4 and IgE responses. (J Allergy Clin Immunol 1998;101:545-51.)

Key words: CD1, CD1b, IgG subclass, IgE, isotype switching, IFN- γ , IL-4

Abbreviations used

APC:	Antigen-presenting cell
CD40 mAb:	Anti-CD40 monoclonal antibody
IFN- γ :	Interferon- γ
LAM:	Lipoarabinomannan
LDN4:	CD1b-restricted double-negative T-cell lines
TCR:	T-cell receptor

The family of CD1 molecules, which are distantly related to major histocompatibility complex class I and class II molecules, are expressed on dendritic cells in various tissues, on mantle zone B cells, and on cytokine-activated monocytes.¹⁻³ Human CD1 exists as five isotypes, CD1a, CD1b, CD1c, CD1d, and CD1e. These molecules are nonpolymorphic and have recently emerged as a third antigen-presenting system that is distinct from either major histocompatibility complex class I or class II molecules.^{1, 4-7} Human CD1b was first shown to act as a restriction element for the presentation of several lipid (mycolic acid) antigens from *Mycobacterium tuberculosis* to human CD4⁺8⁺ T lymphocytes.^{1, 4} Subsequent work extended this finding by showing that CD1b-restricted T cells isolated from the skin lesion of a patient with leprosy specifically respond to an important *Mycobacterium leprae* cell wall constituent, lipoarabinomannan (LAM).⁸ This CD1b-restricted T-cell system requires antigen-presenting cells (APCs) expressing CD1b molecules, internalization of LAM and endosomal acidification in APCs, and CD1-restricted T cells.⁸

Leprosy has provided an excellent model for investigating the role of T cells and cytokines in the human immune response to infection.^{9, 10} The disease forms a spectrum in which clinical manifestations correlate with the level of the immune response to *M. leprae*. Patients with tuberculoid leprosy are able to contain the infection and mount strong T-cell responses to the pathogen. On the other hand, patients with lepromatous leprosy have disseminated infection, and have weak T-cell responses but strong Ig responses to the bacteria.

The goal of this study was to ascertain how CD1-restricted T cells modulate immune responses to microbial pathogens. We used the CD1b-restricted double-negative T-cell line (LDN4) derived from a tuberculoid

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TABLE I. Production of IgG subclass and IgE in CD1b-restricted T-cell system

B cells	APCs	LDN4	LAM	Immunoglobulin production (ng/ml)				
				IgG1	IgG2	IgG3	IgG4	IgE
+	+	+	—	30.6 ± 5.4*	0.5 ± 0.3	56.3 ± 8.9*	13.8 ± 0.3*	10.8 ± 3.2*
+	+	+	+	29.9 ± 7.2*	< 0.1	54.5 ± 3.8*	0.2 ± 0.4	0.3 ± 0.4
+	—	—	—	1.9 ± 0.3	< 0.1	1.1 ± 0.4	< 0.1	< 0.1
+	+	—	—	1.1 ± 0.4	1.0 ± 1.0	0.9 ± 0.8	< 0.1	< 0.1
+	—	+	+	3.5 ± 0.6	< 0.1	4.3 ± 0.6	0.5 ± 0.2	0.8 ± 0.3
+	+	—	+	4.0 ± 1.5	0.8 ± 0.4	8.0 ± 2.1	< 0.1	< 0.1
—	+	+	—	1.7 ± 1.0	0.8 ± 0.5	0.9 ± 0.5	< 0.1	< 0.1
—	+	+	+	1.5 ± 0.4	0.5 ± 0.1	1.2 ± 0.9	< 0.1	< 0.1

Purified IgD⁺ B cells (2×10^5 /well) were incubated in the combination of CD1⁺ APCs (2×10^5 /well), LDN4 (2×10^5 /well), and LAM (1 μ g/ml).

Supernatants were harvested on day 10 for analysis of Ig secretion by ELISA. Data are expressed as mean $\pm$ SD for triplicates.

*Significant difference in Ig production versus nonstimulated B cells alone ($p < 0.01$).

leprosy lesion, which was previously shown to secrete the T_{H1} cytokine pattern.⁸ The ability of LDN4 to modulate humoral immune responses was investigated in terms of Ig subclass production. We found that the CD1b-derived interferon (IFN)- γ can deviate the humoral immune response and thereby may boost pathogen-related cell-mediated immunity.

METHODS

Reagents

The following reagents were used: mouse anti-human IgG (Sigma Chemical Co., St. Louis, Mo.); mouse anti-human IgG1 Fc monoclonal antibody (mAb) (Caltag Laboratories, San Francisco, Calif.); mouse anti-human IgG2 Fc mAb (Caltag Laboratories); mouse anti-human IgG3 Hinge mAb (Caltag Laboratories); mouse anti-human IgG4 Fc mAb (The Binding Site, Ltd., Birmingham, U.K.); alkaline phosphatase-labeled goat anti-human IgG Fab (Tago, Burlingame, Calif.); calibrated standard human IgG1, IgG2, IgG3, and IgG4 (ICN, Irvine, Calif.); mouse anti-human IgE mAb (CIA-E-7.12, CIA-E-4.15);¹¹ alkaline phosphatase-labeled goat anti-human IgE (Kirkegaard and Perry Labs Inc., Gaithersburg, Md.); anti-IFN- γ (Genzyme, Cambridge, Mass.); anti-IL-4 (Genzyme); and granulocyte-macrophage colony-stimulating factor (a gift from Genetics Institute, Cambridge, Mass.). Mouse monoclonal anti-human IgD (mouse IgG) was made from the dTA4-1 hybrid cell line, obtained from American Type Culture Collection (ATCC HB70; Rockville, Md.). IL-4 was the gift of Dr. Eugene Medlock (Amgen, Thousand Oaks, Calif.), CD40 mAb G28-5 was a gift from Dr. Edward A. Clark (University of Washington, Seattle, Wash.), and LAM of *M. leprae* was a gift from Dr. Patrick J. Brennan (Colorado State University, Fort Collins, Colo.).^{12,13}

Cell purification and cell culture

Mononuclear cells were isolated by Ficoll-Hypaque centrifugation from tonsils, removed at surgery for chronic tonsillitis, and further depleted of adherent cells.^{14,15} None of the patients had leprosy or any known exposure to leprosy. Tonsillar B cells were prepared by negative selection by two cycles of 2-aminoethylisothiuronium bromide-treated (Sigma) sheep red blood cells in a rosette formation as described previously.¹⁶ The tonsillar B cells consisted of greater than 95% CD19⁺ cells and less than 1% CD3⁺ cells.^{11,17,18} Resting B cells (IgD⁺ B cells) were then positively isolated with anti-human IgD mouse mAb

and goat anti-mouse IgG-coated magnetic beads (Dynal, Great Neck, N.Y.).^{17,18} This purified population consisted of 95% IgD⁺ B cells and 4% IgD[−] B cells. The purification of the population was assessed by fluorescence analysis on FACScan (data not shown). For stimulation of IgG subclass and IgE production, IgD⁺ B cells (2×10^5 cells/well) were cultured for 10 days.

Adherent cells were obtained from peripheral blood mononuclear cells from healthy volunteers and cultured with granulocyte-macrophage colony-stimulating factor (200 U/ml) and IL-4 (100 U/ml) for 4 days to induce CD1 expression.¹ After stimulation, CD1b expression on adherent cells was determined by fluorescence analysis on FACScan (data not shown). The stimulated adherent cells were used as APCs after the cells were washed three times. The concentration of LAM in the culture system was 1 μ g/ml, a concentration shown to provide for internalization and endosomal acidification of LAM in APCs.⁸

Ig measurements

IgG subclass and IgE levels in the supernatant of stimulated cells were measured by ELISA as previously described.^{11,17,19} Microtiter plates were coated overnight at 4°C with IgG subclass-specific mAbs or anti-human IgE mAbs. After the wells were blocked with 0.1% gelatin, 100 μ l of culture supernatants (undiluted and diluted 1:10, 1:100, and 1:1000) were plated in replicate wells, and the plates were incubated. After washing, either alkaline phosphatase-labeled anti-IgG or IgE was added as the detector. Absorbance at 405 nm was read with an ELISA reader (Bio-Tek Instruments, Inc., Burlingame, Calif.). The sensitivity of the assay for the IgG subclasses and IgE was 0.1 ng/ml. Cross-reactivity to noncorresponding IgG subclasses was not observed in any of the assays (data not shown).

Statistics

Statistical significance for Ig production was determined by a paired *t* test.

RESULTS

Production of IgG subclasses and IgE in the CD1b-restricted T-cell system

The level of IgG subclass and IgE proteins induced by various combinations of IgD⁺ B cells, CD1⁺ APCs, CD1b-restricted T cells (LDN4), and *M. leprae* LAM are

TABLE II. Anti IL-4 mAb is capable of inhibiting production of IgG1, IgG3, IgG4, and IgE in the absence of LAM

LAM	α IL-4	Immunoglobulin production (ng/ml)				
		IgG1	IgG2	IgG3	IgG4	IgE
—	—	20.5 $\pm$ 4.2	0.1 $\pm$ 0.2	44.3 $\pm$ 8.9	15.3 $\pm$ 0.8	14.7 $\pm$ 4.5
—	+	5.8 $\pm$ 4.4*	0.1 $\pm$ 0.5	6.4 $\pm$ 3.2*	<0.1*	< 0.1*
+	—	25.3 $\pm$ 3.2	0.3 $\pm$ 0.3	42.1 $\pm$ 7.4	0.3 $\pm$ 0.3	0.2 $\pm$ 0.1
+	+	22.6 $\pm$ 6.9	0.1 $\pm$ 0.5	44.5 $\pm$ 1.5	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2

Purified IgD⁺ B cells (2×10^5 /well) were incubated with CD1⁺ APCs (2×10^5 /well) and LDN4 (2×10^5 /well) in the presence or absence of LAM (1 μ g/ml), anti IL-4 mAb (1 μ g/ml), or both reagents. Supernatants were harvested on day 10 for analysis of Ig secretion by ELISA.

*Significant difference in Ig production versus in absence of anti IL-4 mAb ($p < 0.01$).

shown in Table I. In the absence of cognate antigen (LAM), the LDN4 is not activated to proliferate in the presence of CD1⁺ APCs or lyse CD1⁺ target cells.⁸ However, in the absence of cognate antigen, LDN4 plus CD1⁺ APCs induced IgG1, IgG3, IgG4, and IgE production from IgD⁺ B cells (Table I, line 1) as compared with IgD⁺ B cells alone (line 3) and IgD⁺ B cells with CD1⁺ APCs (line 4). Notably, IgG2 production was not increased. The addition of *M. leprae* LAM to CD1⁺ APCs activates the CD1-restricted T-cells to proliferate and lyse CD1⁺ target cells.⁸ Although addition of *M. leprae* LAM did induce a marked increase in the level of IgG1 and IgG3 from IgD⁺ B cells (line 2) compared with IgD⁺ B cells alone (line 3), the levels were no higher in cultures without LAM (line 1). Furthermore, in the presence of the CD1 ligand LAM, the production of IgG4 and IgE was markedly (> 95%) inhibited (Table I, line 2). These results were reproducible in four of four independent experiments. Although the APCs did contain B cells capable of making IgG1 and IgG3 (Table I, lines 7 and 8), this could not account for our findings because APCs plus T cells or APCs plus T cells plus LAM failed to increase any IgG subclass and IgE proteins. Similarly, although B cells alone produced a small amount of IgG1 and IgG3 (Table I, line 3), no significant enhancement of any IgG subclass or IgE was observed by IgD⁺ B cells plus LDN4 or IgD⁺ B cells plus APCs.

IL-4-mediated production of IgG4 and IgE in an antigen-nonspecific manner

The mechanism of enhanced IgG1, IgG3, IgG4, and IgE production induced by LDN4 plus APCs plus IgD⁺ B cells in the absence of LAM antigen was suspected to be an effect of IL-4. LDN4 has not been shown to produce detectable levels of IL-4 after incubation with activated APCs in the absence of LAM antigen.⁸ Additionally, LDN4 displays CD40 ligand on its cell surface, and this is increased by stimulation (Sieling et al., unpublished data, 1996). The existence of IL-4 and CD40 ligand could account for the induction of IgG1, IgG3, IgG4, and IgE because we and others have previously shown that IL-4 plus CD40 mAb induces human IgG1, IgG3, IgG4, and IgE isotype switching.^{11, 17, 20, 21} We tested this hypothesis by using anti-IL-4 to determine whether blocking IL-4 would inhibit

IgG1, IgG3, IgG4, and IgE production in the CD1b-nonrestricted cultures. Indeed, anti-IL-4 markedly inhibited Ig production of all the isotypes (Table II). In contrast, the IgG1 and IgG3 production observed in the CD1b-restricted system (in the presence of LAM) was unaffected by anti-IL-4. Thus IL-4 mediates antibody induction by CD1-restricted T cells, although the exact cell source for the IL-4 in the cultures remains to be identified.

Inhibition of IgG4 and IgE in the CD1-restricted system is mediated by IFN- γ

Although unstimulated CD1-restricted T cells or T cells plus CD1⁺ APCs in the absence of LAM produce little IFN- γ , LDN4 T cells secrete large amounts of IFN- γ after incubation with LAM plus CD1-expressing allogeneic APCs.⁸ To confirm the involvement of IFN- γ in the decrease in IgE and IgG4 production observed in the CD1-restricted cultures, anti-IFN- γ mAbs were added to parallel cultures containing LAM. As shown in Fig. 1, in the presence of anti-IFN- γ , production of IgG4 and IgE in the CD1-restricted cultures with LAM was equivalent to that found in the identical cultures without LAM (Table I). Anti-IFN- γ had no effects on production of IgG1, IgG2, and IgG3. Previous studies have shown that anti-CD1b mAbs specifically blocked the release of IFN- γ from LDN4 activated by antigen and APCs.^{1, 8} Thus the failure to produce IgG4 and IgE in the CD1-restricted cultures was primarily an effect of IFN- γ released from LDN4 as a result of CD1 ligation. In the absence of the CD1 ligand LAM, anti-IFN- γ neither enhanced nor inhibited IgG subclass or IgE production (data not shown).

The role of APCs in the induction of IgG1 and IgG3 in the CD1-restricted system

Having shown that both anti-IL-4 and anti-IFN- γ failed to block induction of IgG1 and IgG3 in the CD1-restricted system, we considered whether APCs might be providing the necessary signals driving IgG1 and IgG3 production in the CD1-restricted system. To test the hypothesis that the APCs provided the switch stimulus for CD40-ligated B cells, IgD⁺ B cells were cultured with CD1⁺ APCs plus LAM plus CD40 mAbs (0.1 μ g/ml). As shown in Fig. 2, there was clear induction of IgG1 and IgG3 in the presence of CD40 mAb and in

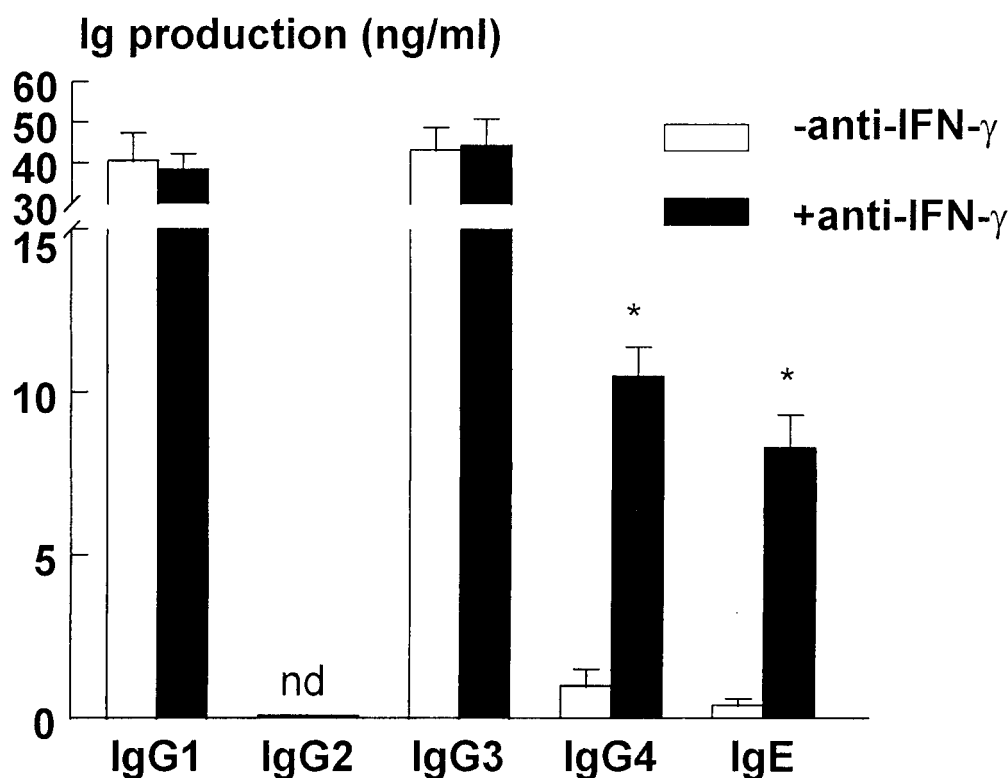


FIG. 1. Inhibition of IgG4 and IgE production by CD1-restricted T cells is mediated by IFN- γ . Purified IgD⁺ B cells (2×10^5 /well) were incubated with CD1⁺ APCs (2×10^5 /well), CD1-restricted T cells (2×10^5 /well), and LAM in absence or presence of anti-IFN- γ (1 μ g/ml). Supernatants were harvested on day 10 for analysis of Ig secretion by ELISA. Data are expressed as mean $\pm$ SD for triplicates. *Significant difference in presence of anti-IFN- γ versus absence of anti-IFN- γ ($p < 0.01$). nd, Not detectable.

the absence of CD1-restricted T cells. Thus the combination of LAM-stimulated CD1⁺ APCs plus CD40L-bearing CD1-restricted T cells appears to account for the induced IgG1 and IgG3 production.

DISCUSSION

In this study we sought to determine whether CD1b-restricted T cells could alter human IgG subclass and IgE production. The LDN4 cell line was derived from the skin of a tuberculoid leprosy lesion. This cell line is a CD4⁺CD8⁻, α/β TCR⁺ and has been shown to be CD1b restricted.⁸ We purposefully used CD1b⁺ APCs plus CD1b-restricted T cells with or without cognate antigen (*M. leprae* or LAM) in the cultures with responding B cells so as to detect either soluble or cell surface ligands that might regulate Ig production. In the absence of LAM, IgD⁺ B cells plus LAM-specific T cells and APCs produced IgG1, IgG3, IgG4, and IgE; isotypes were induced by both IL-4 and CD40 stimulation. In the presence of cognate antigen, we found inhibition of IgG4 and IgE production from IgD⁺ B cells. This downregulation of IgG4 and IgE production was dependent on IFN- γ secretion from the T-cell line, which is consistent with studies indicating that IFN- γ and IL-4 have antagonistic effects as

related to Ig class switching, with IFN- γ inhibiting IL-4 and T-cell mediated human IgE production.²²⁻²⁵ In doing this, IFN- γ appears to act early to prevent switching as it fails to suppress IgE secretion stimulated by CD40 ligation and IL-4.^{26, 27} The ability of the CD1-restricted T-cell line to alter the Ig isotype switching would bias the host response towards cell-mediated immunity.

The addition of anti-IL-4 antibody in the CD1b nonrestricted system inhibited production of IgG1, IgG3, IgG4, and IgE (Table II). Although our CD1b-restricted T cells (LDN4) have not been reported to produce detectable levels of IL-4 after incubation with activated APCs,⁸ there exists the possibility that the necessary amount of IL-4 is released from LDN4 cells after interaction with B cells. Additionally, non-T, non-B cells (e.g., basophils or mast cells) have been reported to be a source of IL-4.²⁸⁻³¹ These cell types, present in low numbers in APC fractions, may be activated by APCs expressing CD1 molecules, T-cell lines, or both and then produce IL-4. The exact cell source of IL-4 in the cultures is under investigation.

A large number of T-cell clones have been established from the blood and skin lesions of patients with leprosy.^{9, 10} These lines generally produce either T_{H1}- or T_{H2}-

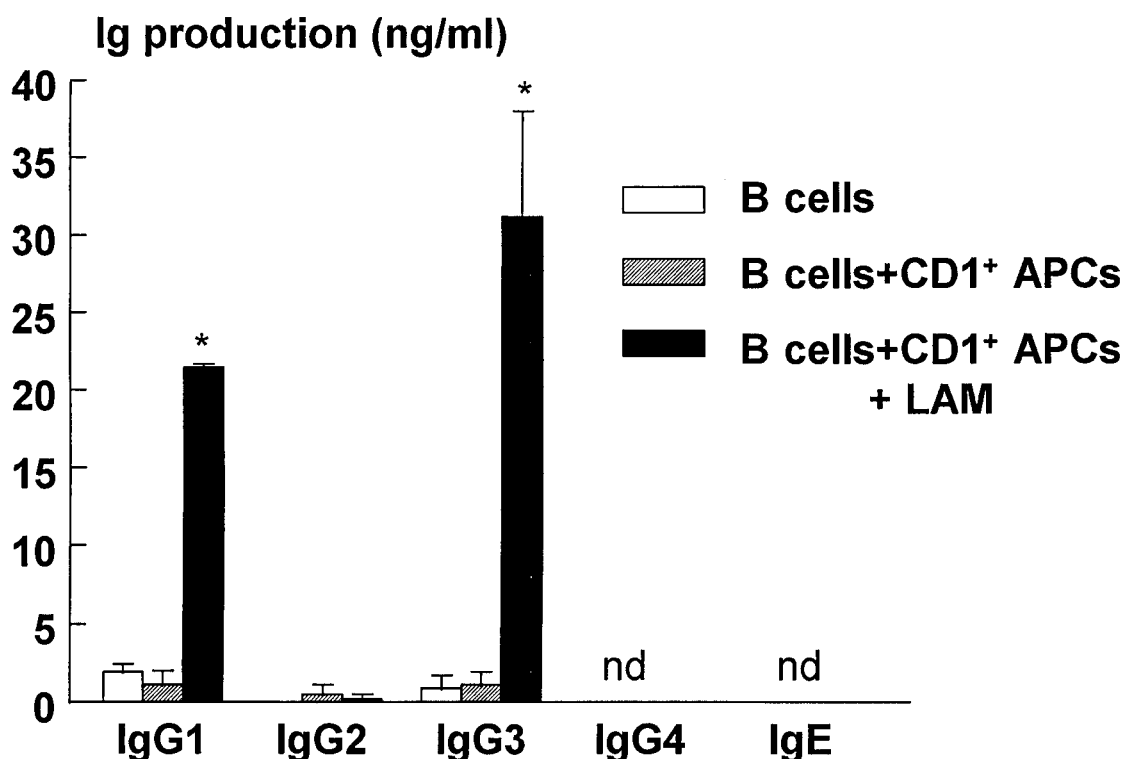


FIG. 2. CD40 activation replaces T-cell signal in CD1-restricted T-cell help for Ig production. Purified IgD⁺ B cells (2×10^5 /well) and CD40 mAb (0.1 μ g/ml) were incubated in presence or absence of CD1⁺ APCs (2×10^5 /well) and LAM. Supernatants were harvested on day 10 for analysis of Ig secretion by ELISA. Data are expressed as mean $\pm$ SD for triplicates. *Significant difference in presence of anti-IFN- γ versus absence of anti-IFN- γ ($p < 0.01$). nd, Not detectable.

type cytokines, depending on the severity of disease.^{9, 10} Stimulation through CD1b could be one trigger regulating the type of secreted cytokines from such T cells. After nonprotein antigen presentation (e.g., LAM through CD1b) the T_{h0}-like quiescent state of CD1b-restricted T cells appears to be changed toward a T_{h1} phenotype by means of IFN- γ , other mediators, or both. This would serve as a rapid way of driving a T_{h1}-like response against infectious organisms that commonly bear such nonprotein antigens on their outer surface.

Previous studies have shown that induction of IgG2 is distinct from that of other IgG subclasses in that IgG2 predominates in the response to polysaccharide-carbohydrate antigens.^{32, 33} The system making use of LAM appears ideal to test the role of the CD1c-restricted cell interactions in driving IgG2 switching. Increased levels of IgG2 to *M. leprae* LAM, compared with the other IgG subclasses, are observed in patients with leprosy.³⁴ IFN- γ , produced by CD1b-restricted LAM-reactive T cells stimulated by their cognate antigen, is known to induce $\gamma 2$ germ-line transcripts from unswitched B cells and enhance IgG2 production from IgG2 committed cells. Yet IFN- γ alone does not drive the actual DNA rearrangement (switch) to IgG2.^{17, 35, 36} In view of the evidence about IgG2 involvement in the response to nonprotein antigens and the nature of the nonprotein

antigens involved in CD1b recognition, we predicted that CD1b triggering of T cells might induce $\gamma 2$ switching. However, the CD1b-stimulated T cells were clearly unable to drive IgD⁺ B cells to switch to $\gamma 2$ in any of our experiments. The actual switch signals for IgG2 remain unknown.

Our study also showed that IgD⁺ B cells produced IgG1 and IgG3 in response to CD40 ligation and culture with APCs plus LAM but in the absence of CD1-restricted T cells. This may result from the presence of increased IL-10, a cytokine demonstrated to be an isotype switch factor specifically for IgG1 and IgG3.^{37, 38} Adherent cell populations such as APCs stimulated with *M. leprae* have been shown to release IL-10.³⁹ Clinical correlates would be the occurrence of high levels of IgG1 and IgG3 antibodies to *M. leprae* in patients with lepromatous leprosy⁴⁰⁻⁴⁴ and the presence of high levels of IL-10 in lepromatous lesions.⁴⁵ Thus the elevation of antigen-specific IgG1 and IgG3 in patients with leprosy may result, at least in part, from the effect of IL-10 released from monocytes stimulated with LAM.

Our results also suggest a potential role for stimulation of CD1b-restricted T cells in modulating IgE and IgG4 humoral immune responses. Selective mucosal activation of type 1 CD1b-restricted T cells might thereby blunt IgE responses through the indi-

rect action of IFN- γ .²²⁻²⁷ Indeed, it has long been known that *Mycobacterium tuberculosis* and its products inhibit IgE responses, an effect that may well be mediated by activation of CD1b-restricted T cells analogous to the cell line we describe. At the same time, it must be remembered that the results we obtained were from a T_{H1}-type CD1b-restricted T-cell line derived from a tuberculoid leprosy lesion, which is felt to represent primarily a T_{H1}-like process. The full range of various types of CD1b-restricted T cells and the ability of CD1b-restricted T cells to alter their functional profiles under differing conditions remain to be elucidated.

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