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B7-1, But Not CD28, Is Crucial for the Maintenance of the CD4⁺ T Cell Responses in Human Leprosy

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We used human leprosy as a model to compare patterns of costimulatory molecule expression in respect to the clinical/immunologic spectrum of disease. We found that B7-1, B7-2, and CD28 transcripts dominated in tuberculoid leprosy patients, who have potent T cell responses to Mycobacterium leprae. In contrast, CTLA-4 was more strongly expressed in lesions from lepromatous patients, who manifest specific T cell anergy to the leprosy bacterium. T cell clones from tuberculoid lesions were CD4⁺CD28⁺ or CD4⁺CD28⁻, and T cell clones from lepromatous lesions were predominantly CD8⁺CD28⁻. The M. leprae-specific recall response of CD4⁺ T cell clones from tuberculoid lesions was blocked by anti-B7-1 mAb, but not by anti-B7-2 mAb or CTLA-Ig. However, anti-CD28 and anti-CTLA-4 mAbs did not block activation of clones from tuberculoid lesions, suggesting that B7-1 may utilize another costimulatory pathway. Peripheral blood T cell responses in the lepromatous form were strongly regulated by CD28 during T cell activation, in contrast to the tuberculoid form. Thus, B7-1 costimulation could play a role in maintaining a strong immune response to the pathogen.

The disease spectrum of leprosy has provided an opportunity to correlate clinical manifestations of human disease with local cytokine patterns and T cell responses. Tuberculoid leprosy patients mount strong cell-mediated immunity to Mycobacterium leprae that limits bacterial growth, whereas in lepromatous leprosy patients, T cells are specifically unresponsive to the pathogen, resulting in progressive infection. Earlier studies demonstrated that tuberculoid leprosy lesions predominantly contain CD4⁺ T cells that secrete type 1 cytokines, including IL-2 and IFN-γ (19, 20). In contrast, lepromatous lesions are characterized by a predominance of CD8⁺ T cells that secrete a type 2 cytokine pattern with prominent expression of IL-4 (19, 20).

Using leprosy as a model, we studied the role of costimulatory molecules in directing effective immunity versus unresponsiveness in the course of the human T cell response to a microbial Ag. We examined whether in vitro restimulation of M. leprae-primed human T cells requires costimulatory signals for their specific activation. Our data indicate that CD28 costimulation was crucial for the proliferation of peripheral blood T cells from patients of the unresponsive lepromatous form of the disease. Furthermore, in vitro secondary activation of human T cell clones derived from tuberculoid skin lesions was largely blocked by anti-B7-1 mAb. Thus, B7-1 was essential for the maintenance of the CD4⁺ T cell-specific responses to M. leprae in the resistant form of human leprosy.

**Materials and Methods**

**Patients**

Patients with leprosy were classified according to the criteria of Ridley and Jopling (21). Skin biopsies were obtained after informed consent from 20 patients, each with either tuberculoid leprosy or lepromatous leprosy. Some specimens were snap frozen in liquid nitrogen after embedding in OCT medium (Ames, Elkhart, IN) and stored at −70°C. Others were used to derive T cell lines and clones specific to M. leprae. Peripheral blood was obtained from 14 other leprosy patients.

**RNA isolation and cDNA synthesis**

Total RNA was isolated from skin biopsy specimens by the method of Chomczynski and Sacchi (22). To facilitate the rapid lysis of the cells isolated from tissue, 40 × 6-μm cryostat sections were added to 4 M guanidinium isothiocyanate buffer. The samples were treated with DNase I (Promega, Madison, WI) for 30 min at 37°C. RNase inhibitor (Boehringer

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Mannheim, Indianapolis, IN) was present during all enzymatic manipulations of RNA. cDNA was synthesized from oligo(dT)-primed RNA by incubation at 42°C with Superscript II reverse transcriptase (Life Technologies, Gaithersburg, MD).

**Polymerase chain reaction**

PCR was performed as previously described (22, 23). Briefly, the PCR reaction mixture contained 2.5 mM MgCl2, 0.2 mM dNTP, 25 pm 5', and 3' oligonucleotide primers, and 2.5 U Taq polymerase (Perkin-Elmer, San Diego, CA). Samples were then amplified in a Gene Amp 9600 Thermo-cycler (Perkin-Elmer) for 35 cycles. Each cycle consisted of denaturation at 94°C for 30 s and annealing/extension at 65°C for 1 min. An aliquot of PCR product was then electrophoresed on 2% agarose gels, transferred to nitrocellulose, and exposed to radiographic film. The sequences of specific primer pairs, 5' to kinase end-labeled oligonucleotide probes, and exposed to radiographic film. After hybridization to kinase end-labeled oligonucleotide probes, and exposed to radiograph film. The sequences of specific primer pairs, 5', 3', and 3' were as follows: β-actin, TGTTGGGCCCAGCGACCA and CTTCCTAATGTCAGC CACGGTTT; B7-1, CTGTTCAGGTTGATACCGGCT and TTTCCAACAGAGGAGGTGAG; B7-2, CTGAAGCTGGCTTGGCCT and CCCTTACTCTCCACCCA; and CTLA-4, TGTTGCCCCAGCTGTCGTTG and GGCACCGTATGACCG. For comparison of B7-1, B7-2, CD28, and CTLA-4 mRNAs, cDNA concentrations were normalized to yield equivalent β-actin PCR products.

**Antigens**

*M. leprae* was provided by R. J. Rees (London, U.K.) and prepared by probe sonication by V. Mehr (Albert Einstein College of Medicine, Bronx, NY). The level of endotoxin in *M. leprae* was measured quantitatively with a Limulus amebocyte lysate assay (Whittaker Bioproducts, Walkersville, MD). Endotoxin in this preparation was <0.1 ng/ml.

**Antibodies**

For FACS analysis, the following Abs were used: CD80, anti-CD86 (Becton Dickinson, San Jose, CA); CD80, anti-B7-2 (Becton Dickinson), murine IgG2b (PharMingen, San Diego, CA); 9.3, anti-CD28, murine IgG2a, biotinylated 11D4, anti-CTLA-4 (24), and murine IgG1 (Bristol Myers Squibb, Seattle, WA); negative controls were isotype-matched primary Abs; positive controls were isotype-matched anti-CD3, anti-CD4, and anti-CD8 primary Abs (PharMingen).

For blocking experiments in proliferation assays, the following Abs were used: CD80, murine IgG1 (Immunotech, Westbrook, ME); CD80, anti-B7-2, murine IgG2b (PharMingen); 9.3, anti-CD28, murine IgG2a, biotinylated 11D4, anti-CTLA-4, anti-CD86, anti-B70/B7-2, and their isotype-matched primary Abs; positive controls were isotype-matched anti-CD3, anti-CD4, and anti-CD8 primary Abs (PharMingen).

**T cell cloning**

CD4+ type 1 T cell clones or cell lines and CD8+ type 2 T cell clones were derived from blood and lesions of tuberculous and lepromatous patients, respectively, as described previously (20). We also studied the CD4+ T cell line LDN-4, which is *M. leprae* specific and CD1 restricted (25). Clones and cell lines were stained every 2 to 3 wk and then expanded twice per week in RPMI 1640 (Life Technologies), 20 mM l-glutamine, 1000 IU/ml penicillin-streptomycin, 200 IU/ml IL-2 (Schiparelli Biostadiums, Fairfield, NJ), 8% heat-inactivated FCS (HyClone, Logan, UT), and 2% heat-inactivated pooled human serum for the CD4+ T cell clones and 10% heat-inactivated pooled human serum for the T cell LDN-4 clones.

**FACS analysis**

Samples of 2 × 105 T cells were stained with purified Ab (2.5 μg/ml) in FACS buffer (5% bovine calf serum/0.01% azide) for 20 min at 4°C. The cells were washed twice, and 30 μl of phycoerythrin-labeled goat F(ab')2, anti-mouse IgG (Cappel, Costa Mesa, CA) or phycoerythrin-labeled streptavidin (Cappel), diluted at 1:100, was added for 20 min at 4°C. After two washes, 5 μl of FITC-labeled mouse anti-CD4 or mouse anti-CD8 (Becton Dickinson) was used to double stain the T cells for 20 min at 4°C, and the cells were washed again twice. The cells were then fixed in FACS buffer/1% paraformaldehyde (Sigma, St. Louis, MO) and analyzed using a FACScan (Becton Dickinson). Data were analyzed and plotted using Becton Dickinson Lysis II software.

**PBMC proliferation and blocking assays**

PBMC were isolated from venous blood following Ficoll-Paque (Pharmacia, LKB Biotechnology, Piscataway, NJ) gradient centrifugation, and were maintained (2 × 105/well) in flat-bottom 96-well plates (Becton Dickinson) at 37°C in a CO2 incubator and cultured in RPMI 1640 (Life Technologies) with 10% heat-inactivated pooled human serum. Sonicated *M. leprae* (5 μg/ml) was added, and cells were grown for 5 days, at which time cells were pulsed with [3H]thymidine (0.5 μCi/well) and harvested 18 to 24 h later (PBD cell harvester; Cambridge Technology, Watertown, MA). [3H]Thymidine incorporation was measured in a liquid scintillation counter. For blocking experiments, anti-B7-1, anti-B7-2, and their isotype controls were used at 5 μg/ml final, and anti-CD28, anti-CTLA-4, CTLA-4 Ig, and their controls were used at 10 μg/ml final. Each point was done in triplicate. Controls included PBMC without Ag, or PBMC with IL-2 as a positive control of proliferation.

**Measurement of Ag-induced proliferation of lines and clones and blocking experiments**

Proliferation of *M. leprae*-specific CD4+ T cell clones and cell lines derived from tuberculous patients was measured at least 8 days after restimulation with Ag and 2 days after feeding with new medium and IL-2. Cells were washed twice and plated in triplicate at 104/well with 3000 rad irradiated DR-matched PBMC from a normal donor (105/well) and *M. leprae* (1 μg/ml) in 96-well plates at a final volume of 200 μl/well. After 3 days, cells were pulsed with [3H]thymidine (0.5 μCi/well) and harvested 4 h later (PBD cell harvester), and [3H]thymidine incorporation was determined. For blocking experiments, anti-B7-1, anti-B7-2, and their isotype controls were used at 5 μg/ml, and anti-CD28, anti-CTLA-4, CTLA-4 Ig, and their controls were used at 10 μg/ml. Controls included CD4+ T cell clones or cell lines plated without Ag, or with DR-mismatched irradiated PBMC, or with IL-2.

**Results**

**Reciprocal expression of B7-1, B7-2, CD28, and CTLA-4 in skin lesions of the polar forms of leprosy**

To investigate the role of costimulatory molecules in resistance or susceptibility to *M. leprae* infection, we used reverse-transcriptase PCR to analyze the pattern of B7-1, B7-2, CD28, and CTLA-4 mRNA expression in cells from skin lesions of 20 leprosy patient individuals (10 tuberculoid and 10 lepromatous). To assure examination of equivalent quantities of cellular mRNA, cDNAs were normalized to yield equivalent β-actin PCR products. We found that B7-1 and B7-2 mRNAs were strongly expressed in lesions from the tuberculoid form (T-lep)5 compared with the lepromatous form (L-lep) (Fig. 1A). CD28, the T cell ligand for B7-1 and B7-2, was more strongly expressed in cells from T-lep lesions (Fig. 1B), while CTLA-4, present equally on human activated CD4+ and CD8+ T cells (24), was expressed at fourfold higher level in cells from lepromatous lesions (Fig. 1B). The altered levels of CD28 and CTLA-4 RNA in cells from tuberculous and lepromatous patients suggest that differential costimulatory molecule expression may contribute to the outcome of leprosy infection.

**CD28 and CTLA-4 surface expression on T cell clones derived from patients with the polar forms of leprosy**

The differential expression of transcripts inciting costimulatory molecules in lesions of tuberculous and lepromatous patients indicates the existence of phenotypically distinct T cell populations in these lesions. However, skin lesions from leprosy patients typically include T lymphocytes as well as monocytes, macrophages, keratinocytes, and dendritic cells. Therefore, to examine phenotypic differences in pure cell populations, we examined the costimulatory molecule profile on T cell clones derived from leprosy patients. Previous studies in our laboratory have shown that in tuberculous leprosy lesions, the CD4+ population predominates, with a CD4:CD8 ratio of 1.9:1, whereas in lepromatous lesions, the CD8 population is in the majority with a CD4:CD8 ratio of 0.6:1 (26, 27). In addition, T cell clones derived from patients with tuberculoid leprosy are CD4+ and produce IFN-γ, characteristic of

5 Abbreviations used in this paper: T-lep, tuberculoid form; L-lep, lepromatous form.
type 1 cells (19, 20). In contrast, T cell clones isolated from patients with lepromatous leprosy are CD8^+ and produce IL-4, characteristic of type 2 cells (19, 20). As described in Table I, seven CD4^+ T cell clones derived from skin lesions of tuberculoid patients and four CD8^+ T cell clones from skin lesions or blood of lepromatous patients were analyzed for cell surface expression of CD28, CTLA-4, B7-1, and B7-2. A representative flow-cytometric analysis of the three types of clones derived from leprosy lesions is shown in Figure 2. The CD4^+ T-lep clones derived from tuberculoid lesions expressed CTLA-4, B7-1, and B7-2 (Table I), showing their activation status under IL-2 culture conditions. Surface expression of CD28 was not detected in two of seven of the CD4^+ T-lep clones, which were derived from the same patient (Table I). Four of four CD8^+ L-lep clones were CD28^- (Table I), as previously observed (28), and described to be suppressor CD8 T cells (29), but these T cell clones expressed CTLA-4, B7-1, and B7-2 (Table I). Stimulation of the CD4^+CD28^- or the CD8^+CD28^- T cell clones by immobilized anti-CD3 for 2 days, or PMA and ionomycin for 4 h did not induce CD28 expression (data not shown).

Specific proliferation of CD4^+ T-lep clones to M. leprae is blocked by anti-B7-1

In primary activation, functional comparison of B7-1 and B7-2, in several murine models or in vitro with human T cells, has indicated variable roles of these costimulatory molecules in T cell activation. However, there are few reports regarding the role of the B7/CD28

### Table I. Phenotype of T cell clones and cell lines derived from leprosy patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clone/Origin</th>
<th>CD28</th>
<th>CTLA-4</th>
<th>B7-1</th>
<th>B7-2</th>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>10.3/lesion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>103.3/lesion</td>
<td>+</td>
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<td></td>
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<td>+</td>
<td></td>
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<td>+</td>
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<tr>
<td>3</td>
<td>103-2E 9/lesion</td>
<td>+</td>
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<tr>
<td>T-lep: type 1 CD4^-CD8^-</td>
<td></td>
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<tr>
<td>4</td>
<td>LDN-4/lesion</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-lep: type 2 CD8^-</td>
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<td>CS300.3/lesion</td>
<td>+</td>
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</table>

^a Measured by flow cytometric analysis with Abs cited in Materials and Methods, as described in the legend to Figure 2.
pathway in the maintenance of T cell-specific responses (9). We used the human CD4+ T cell clones derived from tuberculoid skin lesions in M. leprae proliferation assays in vitro, with simultaneous addition of blocking Abs or CTLA-4 Ig chimeric protein that blocks B7-1 and B7-2 binding (30, 31). We also wanted to examine whether activation of CD4+CD28− T cell clones was dependent on the same costimulatory pathway as CD4+CD28+ T cell clones. The CD8+ T cell clones derived from lepromatous patients did not proliferate to M. leprae. Thus, their costimulation requirement could not be studied for activation in response to the Ag.

In the presence of anti-B7-1 Ab, the specific proliferation of CD4+ T cell clones was partially or totally inhibited in comparison with values obtained in the presence of the isotype control (Fig. 3A). In contrast, anti-B7-2 mAb did not block M. leprae restimulation of the CD4+ T cell clones (Fig. 3A), indicating that this secondary activation was dependent only on B7-1. To confirm the blocking activity of anti-B7-1 mAb, we used a CD1-restricted M. leprae-specific T cell clone. The Ag restimulation of this double-negative CD4+CD8− T cell clone (25) was not inhibited by anti-B7-1 mAb, corroborating the data from Behar et al., which showed that CD4+CD8+CD28−CD1-restricted T cells are not dependent upon B7/CD28 costimulation pathway for activation (32). Thus, the blocking activity of anti-B7-1 mAb on CD4+ T cell clones proliferation was not due to a toxic or nonspecific effect. CTLA-4 Ig failed to inhibit B7-1-dependent T cell proliferation (data not shown). Simultaneous addition of anti-B7-1 and anti-B7-2 mAbs was performed for two CD4+ T cell clones of seven, and blocked the Ag-specific proliferation to the level of anti-B7-1 mAb alone (data not shown). This indicates that B7-1 only is necessary for this secondary activation, and that B7-1 did not synergize with B7-2 for this costimulatory effect.

Anti-CD28- or anti-CTLA-4-blocking Abs did not inhibit the proliferation of the CD4+ T cell clones in the same sets of experiments (Fig. 3B). Furthermore, this specific blocking effect of the anti-B7-1 Ab was observed for the CD4+CD28− as well as for the CD4+CD28+ T cell clones, suggesting the existence of a B7-1-dependent CD28-independent costimulatory pathway.

CD28 cosignal is crucial for stimulation of PBMC from lepromatous patients, in contrast to PBMC from tuberculoid patients

To further explore the role of costimulatory molecules in leprosy, we studied the specific proliferation of PBMC isolated from five patients of the tuberculoid form and five patients of the lepromatous form of the disease, in presence of the same anti-B7-1, anti-B7-2, anti-CD28, and anti-CTLA-4 Abs.

In a representative experiment shown in Figure 4, PBMC from a patient with unresponsive lepromatous leprosy exhibited a lesser...
of patients, blockade of B7-1 or B7-2 molecules significantly in-
formed PBMC from lepromatous patients (Figs. 4 and 5) had either stimulatory or inhibitory effects on proliferation of
M. leprae (Figs. 4 and 5). Identical blocking experiments were performed on PBMC from three patients of the tuber-
culoid form and three patients of the lepromatous form were stimulated with M. leprae in the presence of anti-B7-1, anti-B7-2 mAbs, or CTLA-4 Ig molecules. cpm values were the means of [3H]thymidine incorporation in triplicate well. Results were expressed as the percentage of the mean value obtained in the presence of the isotype-matched negative control or control human Ig: (cpm in the presence of the tested Ab × 100)/cpm in the presence of the negative control). Negative controls had no effect on PBMC proliferation to M. leprae. Because of its variable effects, anti-
CTLA-4 Ab was evaluated with PBMC of four additional lepromatous patients. Each symbol corresponds to an individual patient.

![Image](https://www.jimmunol.org/)

**FIGURE 5.** Complete inhibition by anti-CD28 mAb and by anti-B7 molecules of M. leprae-induced PBMC proliferation of lepromatous pa-
tients. A, PBMC from five patients of T-lep or five patients of L-lep were stimulated with M. leprae in the presence of anti-CD28 (left) or anti-
CTLA-4 (right)-blocking Abs. B, PBMC from three patients of the tuber-
culoid form and three patients of the lepromatous form were stimulated with M. leprae in the presence of anti-B7-1, anti-B7-2 mAbs, or CTLA-4 Ig molecules. cpm values were the means of [3H]thymidine incorporation in triplicate well. Results were expressed as the percentage of the mean value obtained in the presence of the isotype-matched negative control or control human Ig: (cpm in the presence of the tested Ab × 100)/cpm in the presence of the negative control). Negative controls had no effect on PBMC proliferation to M. leprae. Because of its variable effects, anti-
CTLA-4 Ab was evaluated with PBMC of four additional lepromatous patients. Each symbol corresponds to an individual patient.

Discussion

We have demonstrated that in patients with tuberculoid leprosy, whose T cell responses to the pathogen are strong and dominated by type 1 T cells, the B7-1 costimulatory molecule is required for M. leprae-specific T cell recall responses. In patients with the un-
responsive lepromatous leprosy, whose type 2 T cell responses dominate, CD28 was essential for the activation of peripheral blood T cells, in contrast to PBMC from tuberculoid patients. These data indicate that expression of specific pathways of co-
stimulatory molecules could either influence the host response to M. leprae or reflect differences in the initiation of the response to the pathogen.

Earlier studies have demonstrated that in normal donors, B7-1 and B7-2 may deliver qualitatively different signals to human T cells. In vitro, repetitive costimulation with B7-1 results in high levels of type 1 cytokine production, whereas B7-2 costimulation provides an initial signal to induce naive T cells to become IL-4 producers (33). In our present study, we did not find that differen-
tial expression of B7-1 and B7-2 in lesions corresponded with different cytokine patterns, but rather that both B7 costimulatory
molecules were strongly expressed in the responding tuberculoid form of the disease, in which the Th1 response is dominant. In contrast, B7 expression was reduced significantly in lesions of pa-
tients with lepromatous leprosy, in which the Th2 response is dom-
ninant. This increased B7 expression, in tuberculoid lesions, could be a consequence of a Th1 response, as IFN-γ has been shown to up-regulate B7-1 and B7-2 (34). Alternatively, B7-1 and B7-2 could play a role in the regulation of the Th cell response to the pathogen.

We found that B7-1, but not B7-2, was crucial for induction of secondary responses of tissue-derived CD4+ T cells from patients with tuberculoid leprosy. This corroborated the data from van Dijk et al., which demonstrated that in an allogenic system, B7-1 in-
duced T cell proliferation, IL-2, and IFN-γ production more po-
tently than B7-2 (35). In our system, although anti-B7-1 blocked the response of CD4+ T cell clones, neither anti-B7-2-blocking mAb nor the soluble CD28-antagonist CTLA-4 Ig was able to inhibit proliferation. Several isoforms of B7-1 with differential cell type expression have been described, including B7-3/BB-1, which expresses distinct epitopes than B7-1 (36). However, anti-B7-3/BB-1 mAb can cross-react with B7-1 in B7-1-transfected CHO cells (36). It is possible that the anti-B7/BB-1 mAb used in our study recognizes several isoforms of B7-1/B7-3. Thus, the failure of soluble CTLA-4 Ig to inhibit secondary proliferation of the CD4+ T cell clones may be due to the presence of a novel B7-1 isoform.

While B7-1 or a B7-1-like molecule was necessary to maintain the Ag response of CD4+ T cell clones, this secondary response could not be modified by addition of a soluble anti-CD28 mAb. A B7-1-dependent CD28-independent pathway has also been de-
scribed for NK cell-mediated lysis. The B7-1-mediated triggering of NK cytotoxicity could not be blocked by anti-CD28 and
CTLA-4 Abs (37), suggesting the existence of another receptor for B7-1. The existence of a B7-1-dependent CD28-independent path-
way of T cell activation is further substantiated in our report by the study of two CD4+CD28- T cell clones. These two CD4+ T cell

Dependent on B7-1 costimulatory signal. In the lepromatous form, CD28 played a crucial role in the regulation of proliferation of
PBMCs to M. leprae. CTLA-4 was found more expressed in lepromatous lesions, but in vitro functional studies could not defini-
tively determine its role in the induction of specific unresponsiv-
ness to the pathogen in this form of the disease.
clones, derived from the lesion of one tuberculoid patient, lacked CD28, expressed CTLA-4, and proliferated strongly to M. leprae. Moreover, their Ag-specific proliferation was inhibited by the anti-B7-1 mAb. The ability of these clones to respond to Ag in a B7-1-dependent, CD28-independent manner strongly suggests the existence of an alternative B7-1 costimulatory pathway. One hypothesis would be that B7-1 binds to CTLA-4 to promote the secondary activation of the CD4+ T cell clones in response to the Ag. However, in our system, the anti-CTLA-4 mAb we used had no significant effect on Ag-specific proliferation of the CD4+ T cell clones.

B7 costimulatory molecules appear to play a differential role in the activation of skin-derived CD4+ T cell lines and peripheral blood T cells from patients with tuberculoid leprosy. Anti-B7-1 mAb, anti-B7-2 mAb, and CTLA-4g all blocked Ag-specific peripheral blood T cell responses, in contrast to tissue-derived CD4+ T cell lines, in which Ag proliferation was only dependent on B7-1. This differential outcome may be due to the costimulatory requirements of the two cell populations, or of the same population at different stages of activation. Tissue-derived CD4+ T cells from patients with tuberculoid leprosy might form a subpopulation of the peripheral blood circulating Ag-specific T cells. The costimulatory requirements have not been well defined for tissue-derived CD4+ T cells and might differ from those involved in circulating T cells. Alternatively, cell lines and clones that have been stimulated in vitro with Ag may well have differential signaling requirements than primary peripheral blood T cells.

The other evidence for differential regulation of Th cell responses by costimulatory signals in the leprosy model was provided by Ag-specific proliferation assays with PBMC. Addition of an anti-CD28 mAb completely inhibited the specific proliferation of PBMC obtained from patients with the anergic lepromatous leprosy, in contrast to PBMC from tuberculoid patients. The responsive tuberculoid form has been associated with local production of type 1 cytokines, whereas in the anergic lepromatous form, type 2 cytokines dominate in the lesions (19, 20). Our findings corroborate studies in CD4+ deficient mice or in CTLA-4 Ig-treated mice, in which CD28 was required to induce type 2, but not type 1 T cell responses (38–42).

The unresponsiveness of the T cells from lepromatous patients to the pathogen could involve an excessive CTLA-4 signaling, both for the initiation of the immune response and for the recall response to the Ag. To date, CTLA-4 has been described as a physiologic inhibitor of T cell activation (43), but has not been involved in the pathogenesis of any human disease. Recently, potent inhibitory effects of CTLA-4 by inhibition of IL-2 secretion have been demonstrated on resting human CD4+ T cells, in the presence of CD3 and CD28 activation (44). We found that CTLA-4 expression was more strongly expressed in skin lesions from lepromatous patients than in lesions from patients with tuberculoid leprosy. Furthermore, expanded activated T cell clones isolated from lepromatous leprosy lesions expressed a CD8+ CD28+ CTLA-4+ phenotype, and did not proliferate to M. leprae (20, 29). However, addition of CTLA-4 mAb to in vitro proliferation assays could not influence the M. leprae recall response of CD4+ T cell clones from tuberculoid patients. In addition, two CD4+ T cell clones derived from the lesion of one tuberculoid patient lacked CD28 and expressed CTLA-4, but were able to strongly proliferate to M. leprae, suggesting a more complex regulation of the recall response in the tuberculoid form.

We propose a model for the role of costimulatory molecules in the induction of responsiveness versus anergy in human leprosy. It is possible that in the anergic lepromatous form of the disease, the interaction between the MHC class II/Ag complex on APC and the TCR on T cells is not strong enough to induce T cell activation. A second signal delivered through CD28 is essential for T cell proliferation to the pathogen. When the costimulation through CD28 occurs, IL-4 secretion is favored (42), and CTLA-4 expression is augmented, as demonstrated for mouse T cells (45). The IL-4 production and the few CD28 signals still delivered help to maintain the type 2 cytokine secretion (38–42, 46, 47). In addition, CTLA-4 cross-linked by B7-1 and B7-2 then blocks CD28-dependent IL-2 production (16, 17). As a result, the type 2 cytokine dominance is maintained in lepromatous leprosy lesions. Because human CD8+ T cells treated by IL-4 down-regulate CD28 on their surface (48), B7-1 and B7-2 can only bind to CTLA-4, which delivers an inhibitory signal. The CD8+ T cells then have a reduced proliferative capacity (48), as is the case for CD8+ CD28+ T cells isolated from lepromatous patients (20, 49, 50).

In tuberculoid leprosy, the interaction of the MHC class II/Ag complex on APC and the TCR on T cells is strong, such that the CD28 costimulatory signal is not essential for activation. As a result, CTLA-4 is more weakly expressed and does not induce any decrease in IL-2 production, and therefore any type 2 cytokine switch. CD28 is not down-regulated in most of the patients. The proliferative response to the pathogen is strong, and type 1 CD4+ T cells dominate. However, B7-1 seems to be essential to deliver a costimulatory signal to the CD4+ T cells to maintain their activation. Understanding the regulation of T cell activation during recall responses will provide new therapeutic strategies to strengthen immune responses directed against human pathogens.

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References

18. Karandikar, N. J., C. L. Vanderlugt, T. L. Walunas, S. D. Miller, and
31. Linsley, P. S. 1995. Distinct roles for CD28 and cytotoxic T lymphocyte-asso-
27. Modlin, R. L., V. Mehra, L. Wong, Y. Fujimiya, W. C. Chang, D. A. Horwitz,
24. Linsley, P. S., J. L. Greene, P. Tan, J. Bradshaw, J. A. Ledbetter, C. Anasetti, and
29. Li, S. G., T. H. Ottenhoff, P. Van den Elsen, F. Koning, L. Zhang, T. Mak, and