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The generation of cell-mediated immunity against intracellular infection involves the production of IL-12, a critical cytokine required for the development of Th1 responses. The biologic activities of IL-12 are mediated through a specific, high affinity IL-12R composed of an IL-12Rβ1/IL-12Rβ2 heterodimer, with the IL-12Rβ2 chain involved in signaling via Stat4. We investigated IL-12R expression and function in human infectious disease, using the clinical/immunologic spectrum of leprosy as a model. T cells from tuberculoid patients, the resistant form of leprosy, are responsive to IL-12; however, T cells from lepromatous patients, the susceptible form of leprosy, do not respond to IL-12. We found that the IL-12Rβ2 was more highly expressed in tuberculoid lesions compared with lepromatous lesions. In contrast, IL-12Rβ1 expression was similar in both tuberculoid and lepromatous lesions. The expression of IL-12Rβ2 on T cells was up-regulated by Mycobacterium leprae in tuberculoid but not in lepromatous patients. Furthermore, IL-12 induced Stat4 phosphorylation and DNA binding in M. leprae-activated T cells from tuberculoid but not from lepromatous patients. Interestingly, IL-12Rβ2 in lepromatous patients could be up-regulated by stimulation with M. tuberculosis. These data suggest that Th response to M. leprae determines IL-12Rβ2 expression and function in host defense in leprosy.


The outcome of infection in murine models of infection and in human infectious disease is regulated by the Th1 and Th2 T cell cytokine patterns. Th1 cells secrete IL-2 and IFN-γ and are generally associated with resistance to intracellular pathogens, whereas Th2 cells secrete IL-4 and IL-10 and are associated with progressive disease to these same pathogens. It has become increasingly evident that IL-12 is a pivotal regulator of Th1 responses and is essential for promoting cell-mediated immunity (CMI) against intracellular microbial pathogens (1–5).

The ability of IL-12 to activate lymphocytes is mediated by the IL-12R, a heterodimer composed of IL-12Rβ1 and IL-12Rβ2 subunits (6, 7). The IL-12Rβ1 is constitutively expressed on both Th1 and Th2 cells. The IL-12Rβ2, in contrast, is expressed more strongly on Th1 cells as compared with Th2 cells, indicating a mechanism by which IL-12 differentially affects the growth of these subsets. Studies involving the regulation of IL-12R have shown that Ag receptor triggering is sufficient to induce expression of IL-12Rβ1 and IL-12Rβ2 (8). In humans, IL-12Rβ2 chain is induced by IL-12 and IFN-α and inhibited by IL-4 (8, 9), suggesting that local cytokine milieu plays a role in determining IL-12 responsiveness of T cells during infection.

Binding of IL-12 to the IL-12R results in activation of Janus kinases Tyk2 and Jak2, leading to tyrosine phosphorylation and DNA binding of Stat4 with subsequent IFN-γ production by T cells (10, 11). Studies in both human and murine models have shown that IL-12 induces tyrosine phosphorylation of Stat4 in Th1 cells but not in Th2 cells (8, 9, 12, 13), indicating a functional difference imparted by the expression levels of the IL-12R in these T cell subsets.

To study the mechanism of responsiveness to IL-12 in human infectious disease, we chose leprosy as a model because of its spectrum of clinical manifestations that correlate with the level of CMI to the pathogen Mycobacterium leprae. At one end of the spectrum, patients with tuberculoid leprosy mount a strong CMI and are resistant to M. leprae. At the opposite end of the spectrum, patients with lepromatous leprosy have weak CMI and have a progressive form of the disease. Our earlier studies demonstrated that T cells from tuberculoid leprosy patients proliferate in response to IL-12, whereas T cells isolated from lepromatous patients do not respond to IL-12 (14). Here, we present evidence that expression and up-regulation of IL-12Rβ2 correlates with CMI, being greater in tuberculoid than in lepromatous patients. IL-12 signaling in tuberculoid leprosy patients correlates with the expression of the IL-12Rβ2 subunit. The lack of IL-12 responsiveness in lepromatous patients appears to be in part due to an Ag-specific unresponsiveness to M. leprae and due to inappropriately differentiated Th cells. Our data suggest that Th response to Ag determines IL-12Rβ2 expression and function in the generation of CMI to microbial infection.

Materials and Methods

Patients and clinical specimens

Patients with leprosy were diagnosed at the Los Angeles County/University of Southern California Medical Center Hansen’s Disease Clinic (Los Angeles, CA) and classified according to the clinical and pathologic criteria.
of Ridley and Jopling (15). After receiving informed consent, venous blood was collected in heparinized tubes from tuberculous and lepromatous leprosy patients. PBMC were isolated using Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ) differential centrifugation.

Skin biopsies from leprosy patients were also obtained, embedded in OCT medium (Ams, Elkhart, IN), snap-frozen in liquid nitrogen, and stored at −80°C until ready to use.

Antigens

*M. leprae* and *M. tuberculosis* (strain H37Rv) were provided by P. Brennan (Colorado State University, Fort Collins, CO) and prepared by probe sonication (16). The level of endotoxin in *M. leprae* and *M. tuberculosis* was measured quantitatively with a Limulus Amoebocyte Lysate assay (Bio-Whittaker, Walkersville, MD) and found to be <0.1 ng/ml.

Polymerase chain reaction

Total RNA from skin biopsy specimens was isolated by lysing samples in guanidinium isothiocyanate buffer as previously described (17, 18), and cDNA was synthesized with reverse transcriptase (Life Technologies, Gaithersburg, MD).

cDNA samples were amplified in a DNA Thermocycler (Perkin-Elmer, San Diego, CA) in volumes of 10 μl for each cycle consisting of denaturation at 95°C for 20 s and annealing/extension at 65°C for 45 s. Each PCR mixture contained 2.5 μM MgCl₂, 0.2 mM dNTP, 25 pM 5′ and 3′ oligonucleotide primers, and 2.5 U Taq polymerase (Life Technologies, Gaithersburg, MD). The sequences of the primer pairs, 5′ and 3′, were as follows: IL-12Rβ1, 5′-CTGGTTTTAGGACCACCATCC-3′ and 5′-AGAGTTGTAGCACTGCACGACAG-3′; IL-12Rβ2, 5′-GAGGACTCTGACCTGCTTAATGCTTG-3′ and 5′-CCCTCAAGAGCTTATGTTAA-3′; and CD36, 5′-CCTGAAGCTGGGAAAACGCATC-3′ and 5′-GATCTGAGCATCCTCGATC-3′. cDNA concentrations were normalized to yield equivalent CD36 PCR products.

To verify IL-12Rβ1 and IL-12Rβ2 mRNA, PCR products were transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech) as previously described (17, 18) and probed with a labeled oligonucleotide complementary to nucleotides within the sequences recognized by the PCR amplification primers. Sequences of the oligonucleotide probes were as follows: IL-12Rβ1, 5′-GAGATGCTATCGGATATCCAGTGATCG-3′; and IL-12Rβ2, 5′-CTCCCACTATCGGACCTTGCA-3′; and CD36, 5′-GCCGACACACAAGCTCTGTTGAGGA-3′.

The relative intensity of PCR bands was assessed by densitometric analysis of the digitized image, performed on a Macintosh computer (Apple Computers, Cupertino, CA) using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Flow cytometry

PBMC (1 × 10⁶ cells/ml) from tuberculous and lepromatous patients were cultured in complete medium (RPMI 1640, 0.1 mM sodium pyruvate, 2 mM penicillin, 50 μg/ml streptomycin; Life Technologies, Grand Island, NY) supplemented with 10% human serum in a 24-well plate. Cells were stimulated with and without Ag for 5 days. Lymphocytes were recovered, washed twice in PBS, and resuspended in FACS buffer containing PBS with 2% FBS and 0.1% sodium azide. Mononuclear Ab to IL-12Rβ1 and IL-12Rβ2 (provided by D. H. Presky, Hoffmann-LaRoche, Nutley, NJ) or isotype-matched control, IgG2a, (PharMingen, San Diego, CA) was added with 2% FBS and 0.1% sodium azide. Monoclonal Ab to IL-12Rβ1 and IL-12Rβ2 mRNA were measured as fluorescence intensity by FACS (BD Biosciences, Mountain View, CA). The expression of the IL-12R subunits was measured as fluorescence intensity by FACS (BD Biosciences, Mountain View, CA). The expression of the IL-12R subunits was measured as fluorescence intensity by FACS (BD Biosciences, Mountain View, CA). The expression of the IL-12R subunits was measured as fluorescence intensity by FACS (BD Biosciences, Mountain View, CA).

Analysis was performed using a FACScan (BD Biosciences, Mountain View, CA). The expression of the IL-12R subunits was measured as fluorescence intensity by FACS (BD Biosciences, Mountain View, CA).

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The relative intensity of PCR bands was assessed by densitometric analysis of the digitized image, performed on a Macintosh computer (Apple Computers, Cupertino, CA) using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

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**Results**

**IL-12 receptor and leprosy**

**IL-12Rβ1 expression in leprosy lesions correlates with CMI**

To determine the relative expression of IL-12R subunits in leprosy patients, we performed RT-PCR on biopsy specimens from skin lesions of patients with leprosy. RNA was extracted from biopsy specimens, then cDNA was synthesized and normalized to the amounts of CD3-Δ PCR products as a measure of total cellular RNA in T cells. As shown in Fig. 1, the inducible IL-12Rβ2 mRNA was highly expressed in 10 of 10 tuberculoid lesions but only weakly expressed in 10 of 10 lepromatous lesions. In contrast, the constitutively expressed IL-12Rβ1 subunit was equally expressed in both tuberculoid and lepromatous lesions. These data indicate that the expression of IL-12Rβ2 in leprosy correlates with host resistance to infection, greatest in the group of patients known to manifest strong CMI to *M. leprae*.

**IL-12Rβ2 expression correlates with A-g responsiveness in T cells**

Because Ag stimulation has been shown to enhance the expression of the IL-12R on T cells (7), we determined whether addition of *M. leprae* could modulate the expression of IL-12R subunits on T cells from leprosy patients and explain the differential expression in leprosy lesions. PBMC from tuberculous and lepromatous patients were cultured with and without *M. leprae* for 5 days, and the expression of IL-12Rβ1 and IL-12Rβ2 on the cell surface was measured by flow cytometry. When PBMC were cultured with medium alone, the expression of IL-12Rβ1 and IL-12Rβ2 was similar in both tuberculoid (n = 9) and lepromatous (n = 9) patients, with IL-12Rβ1 expression being slightly higher than the
expression of IL-12Rβ2 in both patient groups. Representative examples are shown in Fig. 2a. After stimulation with *M. leprae*, we found that in the tuberculoid patients both IL-12Rβ1 and IL-12Rβ2 were up-regulated. In contrast, in the lepromatous patient group the expression of IL-12Rβ1 but not that of IL-12Rβ2 could be up-regulated by *M. leprae*.

In nine tuberculoid patients, Ag stimulation up-regulated the expression of both IL-12Rβ1 and IL-12Rβ2. The ΔMFI between *M. leprae*-stimulated and unstimulated lymphocytes ranged between 3 and 22 for IL-12Rβ1 (Fig. 2b). For IL-12Rβ2 expression, the ΔMFI varied between 1.6 and 13.3. In lepromatous patients, the IL-12Rβ1 expression was up-regulated by the addition of *M. leprae* in six of nine patients, and the ΔMFI varied between 1.8 and 16.3. The up-regulation of IL-12Rβ2 expression was even less with only five of nine lepromatous patients showing a minimal change in the MFI (<1.1). The ΔMFI for IL-12Rβ1 expression in tuberculoid patients (n = 9) compared with lepromatous patients (n = 9) was not significant (p = 0.07). In contrast, the ΔMFI for IL-12Rβ2 expression between the two groups of patients was statistically significant (p = 0.003). These results suggest that PBLs in tuberculoid patients can respond to Ag stimulation and up-regulate IL-12Rβ1 and IL-12Rβ2. In contrast, the response of lepromatous patients to *M. leprae* is limited to the up-regulation of IL-12Rβ1 expression, but not the up-regulation of IL-12Rβ2 expression.

Although patients with lepromatous leprosy are unable to mount the appropriate CMI response to *M. leprae*, this unresponsiveness is highly specific because these patients have been shown to have intact immune responses to Ags from a variety of other microbial agents, including *M. tuberculosis* (25–27). Therefore, to determine whether the ability to up-regulate IL-12Rβ2 was fundamentally altered in these patients, we asked whether *M. tuberculosis* could up-regulate IL-12Rβ2 expression in lepromatous patients. PBMC from tuberculoid and lepromatous patients were stimulated with either *M. leprae* or *M. tuberculosis* for 5 days, and levels of IL-12Rβ2 expression were determined by FACS. Representative histograms from each patient group are shown in Fig. 3. In tuberculoid patients (n = 3), both *M. leprae* and *M. tuberculosis* induced the expression of IL-12Rβ2 when compared with medium alone. In lepromatous patients (n = 3), *M. leprae* extract had no significant effect on the expression of IL-12Rβ2, but the addition of *M. tuberculosis* extract to culture of PBMC increased the expression of IL-12Rβ2. In summary, although *M. leprae* could not induce IL-12Rβ2 expression in PBMC from the lepromatous patients, activation by *M. tuberculosis* could lead to the up-regulation IL-12Rβ2. These data demonstrate that the inability of lepromatous patients to up-regulate IL-12Rβ2 correlates with their ineffective Th response to *M. leprae*. 

FIGURE 1. IL-12 Rβ1 and IL-12Rβ2 mRNA expression in leprosy lesions. The cDNA derived from the skin lesions of 10 tuberculoid (1–10) and 10 lepromatous (11–20) patients were normalized to yield equivalent CD3-δ mRNA PCR products. PCR with specific primers was then used to detect IL-12Rβ1 and IL-12Rβ2 mRNA. The PCR products were transferred to nylon membrane and probed with a radiolabeled internal oligonucleotide. The intensity of PCR bands was assessed by densitometric analysis of the digitized image using the public domain NIH image program, and is expressed as PCR intensity.
IL-12 induces Stat4-DNA binding in tuberculoid but not in lepromatous patients

The IL-12 signaling pathway involves the activation of the Janus kinase-STAT signal transduction pathway. Specifically, stimulation of the IL-12R by IL-12 leads to the phosphorylation and nuclear translocation of a transcription factor Stat4. Such activation of Stat4 has been shown to be essential for the IL-12-mediated lymphocyte responses (12, 13), and this IL-12 responsiveness appears to correlate well with the expression of IL-12Rβ2 (8, 9). To determine whether the low levels of IL-12Rβ2 expression in lepromatous patients is sufficient for IL-12 signaling, PBMC from tuberculoid and lepromatous patients were stimulated with *M. leprae* and activated with IL-12. Formation of Stat4-containing complexes and binding to the oligonucleotide probe hSIE, which contains a high affinity-binding site for several STAT proteins, were determined by gel shift analysis. As shown in Fig. 4, IL-12 induced binding activity to the hSIE probe in PBMC from a tuberculoid patient (lanes 1–3) and in an IL-12-responsive Th1 clone, D103.5 (lanes 13–15). Arrow points to bands forming Stat4-containing complexes. In contrast, PBMC from a lepromatous patient did not show any Stat4 binding activity (lanes 7–9) upon IL-12 stimulation. Samples without IL-12 stimulation did not demonstrate any binding activity in both tuberculoid (lanes 4–6) and lepromatous (lanes 10–12) patients. Supershift experiments confirmed that these complexes contained Stat4 because Ab to Stat4, but not to Stat3, interfered with this binding. These results suggest that low expression of IL-12Rβ2 in lepromatous patients is not sufficient for IL-12 signaling.

IL-12 induces Stat4 phosphorylation in tuberculoid but not in lepromatous patients

Because the DNA-binding activity of STATs requires tyrosine phosphorylation (28), we next sought to determine whether IL-12 could induce Stat4 phosphorylation in leprosy patients. PBMC from four tuberculoid and four lepromatous patients were cultured with *M. leprae* for 5 days, then stimulated with IL-12. Total cell

**FIGURE 2.** Regulation of IL-12Rβ1 and IL-12Rβ2 expression by *M. leprae*. PBMC (1 × 10⁶) from tuberculoid and lepromatous patients were cultured in vitro in the absence of stimuli (medium) or with *M. leprae* (5 µg/ml) for 5 days. Cells were stained with IL-12Rβ1, IL-12Rβ2, or with an isotype control Ab, followed by PE-conjugated Ab. IL-12Rβ1 and β2 expression was measured on gated lymphocytes. The *M. leprae*-activated T cells are mostly CD4⁺ T cells (25). a, Representative histograms showing the level of IL-12Rβ1 and IL-12Rβ2 expression on T cells from tuberculoid and lepromatous patients. Solid outline, staining with control Ab alone; bold outline, staining with IL-12Rβ1 or IL-12Rβ2 Ab. b, Summary bar graph demonstrating the modulation of MFI of lymphocytes stained with anti-IL-12Rβ1 and anti-IL-12Rβ2 Ab. Nine tuberculoid (BT1-BT9) and nine lepromatous (LL1-LL9) patients were tested. The value indicated is the difference in MFI (∆MFI) in IL-12Rβ1 and 12Rβ2 expression between *M. leprae*-stimulated cells and unstimulated cells.
Activation of T cells by M. tuberculosis samples. Lepromatous samples was not due to the lack of Stat4 in these patients. Anti-Stat4 Ab demonstrated that the lack of phosphorylation in the Stat4 proteins in all four patients (Fig. 5b, lanes 5–8). Our finding suggests that in lepromatous patients, the lack of IL-12 signaling is not likely due to a functional defect in the IL-12Rβ2. Instead, the lack of the IL-12 signaling in these patients may be due to the inability of the lepromatous patients to mount an appropriate Th response to M. leprae, leading to insufficient level of the IL-12Rβ2 expression.

IL-12-induced IFN-γ production correlates with Ag responsiveness in leprosy

Given that IL-12 is pivotal to the generation of a Th1 cytokine response, and particularly IFN-γ production, we studied whether

**FIGURE 5.** M. leprae and M. tuberculosis differentially regulate IL-12-induced Stat4 phosphorylation in leprosy patients. a, M. leprae-activated PBMC (2 × 10⁶) from tuberculoid (lanes 1–4) and four lepromatous (5–8) patients were stimulated with IL-12 (20 ng/ml) for 20 min. Total cell lysates were immunoprecipitated with Stat4 Ab, separated by SDS-PAGE, and blotted with anti-phosphotyrosine and anti-Stat4 Abs. In four of four tuberculoid patients, IL-12 induced tyrosine phosphorylation in samples from all four tuberculoid patients (Fig. 5a, lanes 1–4). In contrast, IL-12 did not induce Stat4 phosphorylation in any of the four samples from the lepromatous patients (Fig. 5a, lanes 5–8). Immunoblotting with anti-Stat4 Ab demonstrated that the lack of phosphorylation in the lepromatous samples was not due to the lack of Stat4 in these samples.
IL-12 responsiveness correlated with the production of IFN-γ. PBMC from tuberculoid and lepromatous leprosy patients were activated with either M. leprae or M. tuberculosis for 5 days and stimulated with IL-12 for 24 h, and the level of IFN-γ in cultured supernatant fluids was determined. As shown in Fig. 6, PBMC from tuberculoid patients (n = 3) activated with M. leprae or M. tuberculosis produced significant levels of IFN-γ upon IL-12 stimulation. In contrast, only M. tuberculosis—but not M. leprae-activated PBMC from lepromatous patients (n = 3) produced significant amounts of IFN-γ upon IL-12 stimulation. Therefore, IL-12 signaling appears to regulate the production of IFN-γ and correlates with IL-12Rβ2 expression in leprosy patients.

Discussion

In this study we investigated the expression and regulation of IL-12R to determine the mechanism of IL-12 responsiveness in patients with leprosy. We found that the IL-12Rβ2 subunit was highly expressed in tuberculoid lesions but only weakly expressed in lepromatous lesions. In contrast, IL-12Rβ1 expression was similar in both tuberculoid and lepromatous lesions. We next determined that in tuberculoid patients, but not in lepromatous patients, the IL-12Rβ2 could be up-regulated by stimulation of T cells with M. leprae. Furthermore, IL-12 induced Stat4 phosphorylation and DNA binding in M. leprae-activated T cells from tuberculoid patients but not from lepromatous patients. The ability of IL-12 to induce signaling correlated with the production of IFN-γ by T cells. These data suggest that IL-12Rβ2 expression and function contribute to CMI and the presence of M. leprae-induced Th1 responses in leprosy.

It has become increasingly evident that IL-12 is a key regulator of Th1 cytokine responses. The ability of IL-12 to stimulate Th1 responses requires the expression of the IL-12R on T cells; however, the two receptor chains are differentially regulated. The IL-12Rβ1 is constitutively expressed on T cells; in contrast, the IL-12Rβ2 is selectively expressed on Th1 but not Th2 cells. In our previous study, we demonstrated that M. leprae-specific T cells from tuberculoid leprosy lesions, which produce the Th1 cytokine pattern, proliferate in response to IL-12, whereas T cells from lepromatous leprosy lesions, which produce the Th2 cytokine pattern, do not (14). Furthermore, IL-12 could not reverse Ag unresponsiveness of PBMC from many lepromatous donors. In our present study, we also found that the loss of IL-12 responsiveness in lepromatous T cells correlated with the loss of IL-12 signaling, as measured by the lack of Stat4 phosphorylation and DNA binding activity. Thus, differential maintenance of IL-12Rβ2 mRNA and cell surface protein expression correlates with the difference in IL-12 responsiveness observed between the two groups of patients. Together these data indicate that M. leprae-induced up-regulation of functional IL-12Rβ2 occurs in tuberculoid but not in lepromatous patients and provide a mechanism for IL-12 responsiveness and unresponsiveness in leprosy.

Several recent studies have demonstrated the importance of IL-12 expression in mouse models and human diseases. IL-12- and IL-12R-deficient mice are immunodeficient, unable to produce cell-mediated immune responses to infection by sublethal doses of Listeria (29). Individuals with mutations in the IL-12R genes have increased susceptibility to infection by Mycobacteria and Salmonella (30, 31). In studying patients with tuberculosis and sarcoidosis, lung T cells were found to express high levels of IL-12Rβ2 in comparison to normal controlled subjects (32). Yet there is a recent report of an IL-12Rβ1-independent pathway of IL-12 responsiveness in human T cells (33). Here we demonstrate that unresponsiveness in lepromatous patients is in part due to insufficient IL-12Rβ2 expression, providing additional evidence for the importance of IL-12R in human infection.

In this study, we did not investigate the differential regulation of IL-12R on CD4+ T cells in comparison to CD8+ T cells, because previous studies have indicated that M. leprae-activated PBMC are mostly CD4+ T cells (25). However, there is evidence that CD4+ and CD8+ T cells regulate IL-12R expression by distinct mechanisms. A recent study by Elluso et al. (34) demonstrated that CD28 regulates IL-12Rβ1 expression in CD4+ T cells but not in CD8+ T cells. Therefore, it would be important to study the regulation of IL-12R expression and function in different T cell subsets. Additional studies are needed to clarify the regulation of IL-12R in CD4+ and CD8+ T cells.

Although the lepromatous patients lack the appropriate CMI response to M. leprae, most of these patients exhibit normal CMI to a variety of bacterial Ags, including M. tuberculosis (25–27). Our results further support these previous findings because PBMC from lepromatous patients exhibited intact IL-12 responsiveness when cells were initially stimulated with M. tuberculosis. M. tuberculosis-activated lymphocytes derived from lepromatous patients demonstrated increased IL-12Rβ2 cell surface expression on T cells. Furthermore, intact IL-12 signaling was demonstrated by the phosphorylation of Stat4 in M. tuberculosis-activated cell lysates. These data suggest that lepromatous patients do not have a genetic defect in the structure or function of the IL-12R but rather they are unable to respond to M. leprae and mount appropriate Th response to up-regulate the IL-12R expression.

The mechanism behind the intact IL-12 signaling in T cells from lepromatous patients stimulated with M. tuberculosis vs M. leprae appears to be dependent on the T helper differentiation state. Human and animal studies have demonstrated that Th1 cells up-regulate IL-12Rβ2 whereas Th2 cells do not. In addition, intact IL-12 responsiveness to Ag occurs in Th1 cells and not in Th2 cells (8, 9). Similarly, T cells from lepromatous
patients produce IFN-γ (35) when stimulated with *M. tuberculosis* Ags, and our data demonstrate that these Th1 cells have intact IL-12R function. In contrast, when T cells from lepromatous patients are stimulated with *M. leprae* sonicate, there is little IFN-γ production (14); we show here that these Th2 cells do not respond to IL-12. Therefore, our findings support previous studies because Th1/Th2 differentiation appears to regulate IL-12R expression and function in leprosy.

Exactly what factors influence Th1/Th2 differentiation remains unclear. The dose of Ag, route of Ag delivery, and genetic components including HLA haplotype influence Th1/Th2 development. One of the most clearly defined factors that influences Th1/Th2 pathway are cytokines present at the initiation of the immune response at the ligation of the TCR.

Two cytokines, IL-12 and IL-4, have been implicated as the critical inducer of the Th1 and Th2 pathway, respectively. IL-12 responsiveness may depend on these local cytokines to modulate the expression of the IL-12R. During the Th2 development in BALB/c mice, early IL-4 production induced upon *Leishmania major* infection is thought to be responsible for the down-regulation of the IL-12Rβ2 (36). In human leprosy, a similar mechanism may lead to the blocking of IL-12 signaling seen in lepromatous patients. However, the presence of IL-4 does not appear to be the primary mechanism behind the down-regulation of IL-12Rβ2 expression, because a previous study from our laboratory found low levels of IL-4 when T cells from lepromatous patients were stimulated with *M. leprae* in vitro (14). Recently a number of Th1/Th2 specific transcription factors have been identified. GATA-3 is a Th2-specific transcription factor that appears to promote IL-5 and IL-13 production (37, 38). Furthermore, GATA-3 has been shown to inhibit IFN-γ production through down-regulation of IL-12R expression, thus leading T cells to IL-12 unresponsiveness (39). Whether GATA-3 is involved in down-regulation of the IL-12Rβ2 and JAK kinases: evidence for antigen presentation by a second member of the human CD1 family. J. Immunol. 157: 2795.

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