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A Role for IL-12 Receptor Expression and Signal Transduction in Host Defense in Leprosy

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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.

In 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.


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1 Division of Dermatology, and 2 Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles, CA 90095; 3 Section of Dermatology, University of Southern California School of Medicine, Los Angeles, CA 90033; and 4 Division of Hematology-Oncology, University of California School of Medicine, Los Angeles, CA 90095

2 Address correspondence and reprint requests to Dr. Robert L. Modlin, University of California, Division of Dermatology, 52-121 Center for Health Sciences, 10833 Le Conte Avenue, Los Angeles, CA 90095. E-mail address: rmmodlin@mednet.ucla.edu

3 Abbreviations used in this paper: CMI, cell-mediated immunity; MFI, mean fluorescence intensity; hSIE, high affinity serum-inducible element.

4 Division of Dermatology, and 5 Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles, CA 90095; 6 Section of Dermatology, University of Southern California School of Medicine, Los Angeles, CA 90033; and 7 Division of Hematology-Oncology, University of California School of Medicine, Los Angeles, CA 90095

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3 Address correspondence and reprint requests to Dr. Robert L. Modlin, University of California, Division of Dermatology, 52-121 Center for Health Sciences, 10833 Le Conte Avenue, Los Angeles, CA 90095. E-mail address: rmmodlin@mednet.ucla.edu

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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 tuberculoid 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 (Ames, Elkhart, IN), snap-frozen in liquid nitrogen, and stored at −80°C until ready to use.

Antigens

*M. lepraee* 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. lepraee* 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) for 30 cycles with 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 mM MgCl₂, 0.2 mM dNTP, 25 pM 5′ and 3′ oligonucleotide primers, and 2.5 U Taq polymerase (Life Technologies, Grand Island, NY). The sequences of the primer pairs, 5′ and 3′, were as follows: IL-12Rβ1, 5′-CTGTTTCAAGGACCCGCTATCC-3′ and 5′-AGATGTTGACAGTGCAACGACAG-3′; IL-12Rβ2, 5′-GGAGCTGCTGACTCTTATAGTCG-3′ and 5′-CCCTCCAGAGCTGCAATTTGA-3′; and CD36, 5′-CTGAGCAGTGGAAGAGCGGC-3′ and 5′-GTATCGAGCATCTCATGTCAT-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 probe. Blots were then stripped and reprobed with anti-Stat4 Ab.

Immunoprecipitation and SDS-PAGE

Total cellular lysates of 2 × 10⁷ cells were immunoprecipitated with anti-Stat4 Ab as previously described (24) and were separated by electrophoresis on 8% SDS-PAGE. After transfer to nitrocellulose, blots were probed with anti-phosphotyrosine Ab, 4G10 (Upstate Biotechnology, Lake Placid, NY). Blots were then stripped and reprobed with anti-Stat4 Ab.

IFN-γ production

Ninety-six-well ELISA plates (Corning Glass Works, Corning, NY) were coated overnight at 4°C with 100 μl of mouse anti-human IFN-γ (Pharmingen) at 5 μg/ml per well. Plates were blocked with 200 μl of 1% BSA and 0.05% Tween 20 in PBS for 2 h at room temperature. Aliquots (100 μl) of each sample or of IFN-γ standards (Endogen, Woburn, MA) were then added to each well and incubated at room temperature for 2 h. Biotin-conjugated mouse anti-human IFN-γ Ab (Pharmingen) was added to each well and incubated for 1 h, followed by a 30-min incubation with streptavidin-peroxidase (Pierce, Rockford, IL). Peroxidase substrate solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used to detect IFN-γ, and the plates were read at a wavelength of 405 nm in a 7520 microplate reader (Cambridge Technology, Cambridge, MA). All samples and standards were performed in duplicate.

Results

IL-12 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-β 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 equall 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 Ag 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 tuberculoid 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-12Rβ2 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 the 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.
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-12R (8, 9). To determine whether the low levels of IL-12R 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 oligonuclear 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 (lanes 3 and 15), but not to Stat3 (lanes 2 and 14), interfered with this binding. These results suggest that low expression of IL-12R 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 (ΔMFI) in IL-12Rβ1 and 12Rβ2 expression between M. leprae-stimulated cells and unstimulated cells.
Activation of T cells by M. tuberculosis activates Stat4 phosphorylation in lepromatous patients. Stat4 phosphorylation was measured by immunoblotting with anti-Stat4 Ab. 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...
IL-12 responsiveness correlated with the production of IFN-γ. PBMC from tuberculosis 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 was determined by ELISA. IFN-γ levels are expressed in nanograms per milliliter. ■ M. leprae-activated cells; □ M. tuberculosis-activated cells.

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-12-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-12 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 Elloso 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 Th1 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 Th 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 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 infection, 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 be downregulated in lepromatous patients (40), may direct IL-12 responsiveness. Additional studies to elucidate factors that control the expression and function of IL-12R will be important in developing effective strategy in combating human infectious disease.

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