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*J Immunol* 1999; 162:2441-2447; http://www.jimmunol.org/content/162/4/2441

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Expression of the IL-12 Receptor β1 and β2 Subunits in Human Tuberculosis

Ming Zhang,* Jianhua Gong,* David H. Presky,† Wanfen Xue,‡ and Peter F. Barnes*§¶

To determine whether the Th1 response in tuberculosis correlated with IL-12R expression, we measured expression of the IL-12Rβ1 and IL-12Rβ2 subunits, as well as IL-12Rβ2 mRNA expression in tuberculosis patients and healthy tuberculin reactors. In tuberculosis patients, IFN-γ production by Mycobacterium tuberculosis-stimulated PBMC was reduced, the percentages of T cells expressing IL-12Rβ1 and IL-12Rβ2 were significantly decreased, and IL-12Rβ2 mRNA expression was also markedly reduced. In contrast, in pleural fluid and lymph nodes at the site of disease in tuberculosis patients, in which IFN-γ production is enhanced, IL-12Rβ2 mRNA expression was also increased. In M. tuberculosis-stimulated peripheral blood T cells from tuberculosis patients, anti-IL-10 and anti-TGF-β enhanced IL-12Rβ1 and IL-12Rβ2 expression, and IFN-γ production. In M. tuberculosis-stimulated peripheral blood T cells from healthy tuberculin reactors, recombinant IL-10 and TGF-β reduced IL-12Rβ1 and IL-12Rβ2 expression, as well as IFN-γ production. In combination with prior studies showing increased production of TGF-β by blood monocytes from tuberculosis patients, this suggests that increased TGF-β production is the underlying abnormality that reduces IL-12Rβ1 and IL-12Rβ2 expression in tuberculosis. Our findings provide evidence that IL-12R expression correlates well with IFN-γ production in human tuberculosis, and that expression of IL-12Rβ1 and IL-12Rβ2 may play a central role in mediating a protective Th1 response. The Journal of Immunology, 1999, 162: 2441–2447.

There are at least two compelling reasons to study the human immune response to Mycobacterium tuberculosis. First, tuberculosis is the leading cause of death from infectious agents worldwide, claiming 3 million lives in 1995 and a projected 3.5 million in the year 2000 (1). Tuberculosis control efforts in developing countries are hampered by the high cost of antituberculosis drugs, difficulty in ensuring completion of prolonged therapy, and increasing rates of drug resistance. Prevention of tuberculosis through vaccination is a cost-effective strategy that would contribute greatly to tuberculosis control. Development of a vaccine hinges on an improved understanding of the immune response against M. tuberculosis.

The second reason to study tuberculosis is that it provides an excellent model to investigate the relationship between the immune response and clinical manifestations of disease from intracellular pathogens. Most persons infected with M. tuberculosis are healthy tuberculin reactors who develop protective immunity. Patients with active tuberculosis have severe disease and ineffective immunity, and M. tuberculosis-stimulated PBMC from tuberculosis patients show depressed production of the Th1 cytokine IFN-γ, compared with healthy tuberculin reactors (2, 3). Elucidation of the mechanism for this reduced Th1 response will enhance our understanding of resistance and susceptibility to disease from intracellular pathogens, such as viruses, fungi, and protozoa, as strong Th1 responses are central to immunity against these organisms (4–8).

Murine and human Th1 clones express mRNA for the IL-12Rβ2 subunit, whereas Th2 clones do not (9, 10). In addition, resistance to Leishmania major infection in mice correlates with increased expression of IL-12Rβ1 and IL-12Rβ2 (11). To determine whether the reduced systemic Th1 response in tuberculosis is related to IL-12R expression, we measured IL-12R protein and mRNA expression in peripheral blood and at the site of disease in patients with tuberculosis.

Materials and Methods

Patient population

Blood was obtained from 9 healthy tuberculin reactors and from 27 HIV-seronegative patients with culture-proven pulmonary tuberculosis who had received less than 2 wk of antituberculosis therapy. By standard chest radiographic criteria, 5 patients had moderately advanced disease, and 22 patients had far advanced disease. Acid-fast stains of sputum were positive in all patients.

Frozen cervical lymph node tissue samples were obtained from five patients whose lymph nodes showed benign follicular hyperplasia and from five HIV-seronegative patients with tuberculous lymphadenitis who had received less than 4 wk of antituberculosis therapy. Pleural fluid and blood were obtained from four patients with tuberculosis pleuritis who had received less than 2 wk of antituberculosis therapy.

Abs to IL-12R subunits

The 2B10 Ab to human IL-12Rβ1 (12) was used. In addition, hybridomas producing anti-human IL-12Rβ2 were made by fusing SP2/0 myeloma cells with splenocytes isolated from Lewis rats immunized and boosted with purified recombinant human IL-12Rβ2-IgG1 fusion protein. A mAb 2B6 was generated, which binds to the surface of a transfected Ba/F3 cell line that expresses human IL-12Rβ2 (13), but not to the parental Ba/F3 cell line or to transfected Ba/F3 cells that express human IL-12Rβ1 (D.H.P., unpublished data). Purified 2B6, a rat IgG2a Ab, was produced from ascitic fluid by sequential caprylic acid and ammonium sulfate precipitation (14).

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Received for publication August 28, 1998. Accepted for publication November 10, 1998.

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1 This work was supported by the National Institutes of Health (AI27285, AI36069), P.F.B. holds the Margaret E. Byers Cain Chair for Tuberculosis Research. Mycobacterium tuberculosis vaccination in the year 2000 (1). Tuberculosis control efforts in developing countries are hampered by the high cost of antituberculosis drugs, difficulty in ensuring completion of prolonged therapy, and increasing rates of drug resistance. Prevention of tuberculosis through vaccination is a cost-effective strategy that would contribute greatly to tuberculosis control. Development of a vaccine hinges on an improved understanding of the immune response against M. tuberculosis.

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Cell culture conditions
PBMC and pleural fluid mononuclear cells were isolated by differential centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ). For patients with tuberculous pleuritis, pleural fluid cells and PBMC were lysed with 4 M guanidinium isothiocyanate and stored at 20°C before preparation of RNA. For patients with pulmonary tuberculosis and for healthy tuberculin reactors, PBMC were isolated by differential centrifugation over Ficoll-Paque (PharMingen, San Diego, CA). In some experiments, recombinant IL-10 (10 ng/ml; Genzyme) or TGF-β (Caltag Laboratories, Burlingame, CA) were also added to the cell cultures. In some experiments, primers specific for IL-2Rβ and IFN-γ were also used. The 5’ and 3’ primers, respectively, were as follows: IL-12Rβ2, GAG GGA CTG GTA CTG CTT AAT CGA CTG; and CTC CCT ACA GGT TCA TTA TGT AAT TAC GAG TG; IL-2Rγ, CCA CTC TGT GGA AAT CTA and TCC GTT CCA GCC AGA AAT AC; and IFN-γ, AGT TAT ATC TTG GCT TTT CAG CTC TGC and CCT CAC CGA ATA ATT AGT CAG CTT TTC. cDNA was amplified by 35 cycles of denaturation at 94°C for 1 min and annealing/extension at 65°C for 2 min. For IL-12Rβ2 cDNA, the predicted size of the target PCR product was 511 bp and that of the standard PCR product was 367 bp. For IL-2Rγ cDNA, the predicted size of the target PCR product was 180 bp, and that of the standard PCR product was 322 bp. PCR product was quantified by competitive PCR, as outlined above for CD3. Positive controls containing cytokine receptor cDNA (from PHA-stimulated PBMC) and negative controls containing no cDNA were employed in each set of reactions.

Statistical analysis
Data for different groups were expressed as the mean ± SEM, and were compared by the paired or unpaired Student’s t test, as appropriate. For data that were not normally distributed, the Wilcoxon rank-sum test was used.

Results
Expression of IL-12R in M. tuberculosis-stimulated T cells
In freshly isolated PBMC from healthy tuberculosis reactors, 20–30% of cells were IL-12Rβ1+, compared with only 2–3% of PBMC from tuberculosis patients. In both groups, the percentages of IL-12Rβ1+ cells increased in parallel during 5 days of culture (Fig. 2A). There was no significant IL-12Rβ2 expression in freshly isolated PBMC from healthy tuberculosis reactors or tuberculosis patients (Fig. 2B). IL-12Rβ2 expression was similar in both groups for the first 3 days, but increased more markedly in healthy tuberculosis reactors after day 3. Subsequent measurements of IL-12R expression for M. tuberculosis-stimulated PBMC were not made after 5 days of culture because cell viability decreased after this time point.

In M. tuberculosis-stimulated PBMC from seven tuberculosis patients, the percentage of IL-12Rβ1+ cells was significantly lower than that in cells from seven healthy tuberculosis reactors (mean 18 ± 8 versus 46 ± 6, p = 0.01; Fig. 3). Similarly, the percentage of IL-12Rβ2+ cells was lower in tuberculosis patients (10 ± 3 versus 37 ± 3, p < 0.0001). Expression of IL-12Rβ1 and IL-12Rβ2 paralleled IFN-γ production, as mean IFN-γ concentrations in supernatants of M. tuberculosis-stimulated PBMC from tuberculosis patients were lower than corresponding values in healthy tuberculosis patients.

To accurately compare cytokine mRNA expression in different specimens, it is critical to use equivalent amounts of substrate cDNA. Because we were interested in measuring IL-12Rβ2 mRNA expression by T cells, we normalized all samples for CD3 cDNA content by competitive PCR, as previously described (3). Briefly, the target CD3 sequence and a competitor DNA construct were coamplified by the 5’ primer CTG GAC CTG GGA AAA CGC ATC and the 3’ primer GAC ATG AGC ATC ATC TCG ATC. A DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) ran 26 cycles of denaturation at 94°C for 1 min and annealing/extension at 65°C for 2 min. PCR product (10 μl) was subjected to electrophoresis on 1.5% agarose gels and visualized by staining with ethidium bromide. For quantifying PCR product, gels were photographed with a SPEEDLIGHT gel documentation system (B/T Scientific Technologies, Carlsbad, CA) and analyzed with QGEL software (Kendrick Laboratories, Madison, WI), an imaging and analysis system that permits accurate comparison of the integrated density of the PCR product bands for target and MIMIC cDNA. By plotting the ratio of integrated density of sample to MIMIC cDNA product against the known amount of MIMIC substrate cDNA, the amount of cDNA in each sample was calculated. This method allows accurate detection of twofold differences in substrate cDNA concentrations (3).

For each sample, aliquots containing equivalent amounts of CD3 cDNA were used as substrate and amplified by competitive PCR with primers specific for IL-12Rβ2. In some experiments, primers specific for IL-2Rγ and IFN-γ were also used. The 5’ and 3’ primers, respectively, were as follows: IL-12Rβ2, GAG GGA CTG GTA CTG CTT AAT CGA CTG; and CTC CCT ACA GGT TCA TTA TGT AAT TAC GAG TG; IL-2Rγ, CCA CTC TGT GGA AAT CTA and TCC GTT CCA GCC AGA AAT AC; and IFN-γ, AGT TAT ATC TTG GCT TTT CAG CTC TGC and CCT CAC CGA ATA ATT AGT CAG CTT TTC. cDNA was amplified by 35 cycles of denaturation at 94°C for 1 min and annealing/extension at 65°C for 2 min. For IL-12Rβ2 cDNA, the predicted size of the target PCR product was 511 bp and that of the standard PCR product was 367 bp. For IL-2Rγ cDNA, the predicted size of the target PCR product was 180 bp, and that of the standard PCR product was 322 bp. PCR product was quantified by competitive PCR, as outlined above for CD3. Positive controls containing cytokine receptor cDNA (from PHA-stimulated PBMC) and negative controls containing no cDNA were employed in each set of reactions.
healthy tuberculin reactors (433 ± 105 pg/ml versus 3132 ± 260 pg/ml, p < 0.0001).

Expression of IL-12R by T cell subpopulations in M. tuberculosis-stimulated T cells

To evaluate IL-12R expression in T cell subpopulations, we performed single and double immunolabeling with anti-IL-12Rβ1 or anti-IL-12Rβ2, in combination with anti-CD3, anti-CD4, and anti-CD8. In M. tuberculosis-stimulated PBMC, the percentages of CD3+ cells were similar in tuberculosis patients and healthy tuberculin reactors (69 ± 6 versus 78 ± 6, p > 0.05), as were the percentages of CD4+ and CD8+ cells (data not shown). In three healthy tuberculin reactors and six tuberculosis patients, the percentages of CD3+ cells that expressed IL-12Rβ1 and IL-12Rβ2 were depressed in tuberculosis patients (Fig. 4, p = 0.003 and 0.001, respectively). The percentages of CD4+ cells expressing IL-1Rβ1 and IL-1Rβ2 were also depressed in tuberculosis patients (p = 0.02 and p = 0.0001, respectively). In contrast, the percentages of CD8+ cells expressing IL-12Rβ1 and IL-12Rβ2 were...
To determine whether depressed expression of IL-12R in M. tuberculosis-stimulated PBMC of tuberculosis patients was mediated by changes in transcription or stability of IL-12R mRNA, we determined mRNA levels by competitive RT-PCR. Amplification of IL-12Rβ1 cDNA was unsatisfactory, despite the use of multiple primer pairs and reaction conditions. However, amplification of IL-12Rβ2 cDNA was achieved. Consistent with the data obtained by immunolabeling, IL-12Rβ2 mRNA was undetectable or expressed at very low levels in freshly isolated PBMC from tuberculosis patients or from healthy tuberculin reactors (data not shown). After stimulation of PBMC with M. tuberculosis, T cells were isolated by immunomagnetic selection and IL-12Rβ2 mRNA was determined (Fig. 5). IL-12Rβ2 mRNA expression was greatly reduced in 9 of 11 tuberculosis patients (mean 0.30 ± 0.14 × 10⁻³ attoM, compared with 1.5 ± 0.3 × 10⁻³ attoM in healthy tuberculin reactors, p < 0.001). Reduced expression of IL-12Rβ2 mRNA did not reflect generalized depression of cytokine receptors in tuberculosis, as expression of IL-2Rγ mRNA was not different in tuberculosis patients and healthy tuberculin reactors (Fig. 5).

Expression of IL-12Rβ2 mRNA at the site of disease in vivo

Although the Th1 response is depressed in the peripheral blood of tuberculosis patients, IFN-γ mRNA and protein are selectively increased in lungs, lymph nodes, and pleural fluid of patients with tuberculosis (16–18). Competitive RT-PCR confirmed increased expression of IFN-γ mRNA in lymph nodes from tuberculosis patients, compared with those showing benign follicular hyperplasia (Fig. 6). To determine whether changes in IL-12Rβ2 paralleled those of IFN-γ, we evaluated IL-12Rβ2 mRNA expression at the site of disease. In lymph nodes from five tuberculosis patients, IL-12Rβ2 mRNA expression was 50-fold higher than in lymph nodes from patients with benign follicular hyperplasia (Fig. 6, mean of 6.9 ± 2.6 × 10⁻³ attoM for tuberculosis versus 0.14 ± 0.03 × 10⁻³ attoM for benign follicular hyperplasia, p = 0.01, Wilcoxon rank-sum test). In contrast, expression of IL-2Rγ mRNA was similar in lymph nodes from patients with or without tuberculosis (Fig. 6).

To confirm that IL-12Rβ2 mRNA expression was increased at the site of disease, compared with the peripheral blood of the same patients, we determined IL-12Rβ2 mRNA expression in freshly isolated pleural fluid mononuclear cells and PBMC from four patients with tuberculous pleuritis (Fig. 7). IL-12Rβ2 mRNA expression was greater in pleural fluid cells (mean 0.37 ± 0.07 × 10⁻³ attoM, compared with 0.13 ± 0.06 × 10⁻³ attoM for PBMC). Expression of IL-2Rγ mRNA was similar in pleural fluid and blood (Fig. 7). In confirmation of previous work (18), IFN-γ mRNA expression was also greater in pleural fluid cells (mean 20.2 ± 2.2 × 10⁻³ attoM versus 5.6 ± 0.3 × 10⁻³ attoM for PBMC).

Effects of IL-10 and TGF-β on IL-12R expression

In response to M. tuberculosis, mononuclear phagocytes produce IL-10 and TGF-β (15, 19, 20), both of which inhibit IFN-γ production in response to mycobacterial Ags (21–24). IL-12 enhances

**FIGURE 5.** Expression of IL-12Rβ2 mRNA in M. tuberculosis-stimulated T cells from healthy tuberculin reactors (PPD+) and tuberculosis patients. PBMC from 9 healthy tuberculin reactors and 11 tuberculosis patients were cocultured with heat-killed M. tuberculosis. After 2 days, T cells were isolated, samples were normalized for CD3 cDNA content, and mRNA expression for IL-12Rβ2 and IL-2Rγ was determined by competitive RT-PCR. In each panel, the far left lane shows m.w. markers.

**FIGURE 6.** Expression of mRNA for IL-12Rβ2, IFN-γ, and IL-2γ in lymph nodes from patients with tuberculosis (TB) and from control patients without tuberculosis. Lymph nodes were obtained from five tuberculosis patients and from five healthy persons without tuberculosis. After normalization for CD3 cDNA content, mRNA expression for IL-12Rβ2, IFN-γ, and IL-2γ was determined by competitive RT-PCR. In each panel, the far left lane shows m.w. markers.

**FIGURE 7.** Expression of mRNA for IL-12Rβ2, IFN-γ, and IL-2γ in freshly isolated pleural fluid mononuclear cells and PBMC from patients with tuberculous pleuritis. Pleural fluid mononuclear cells (PF) and PBMC (PB) were obtained from four patients with tuberculous pleuritis. After normalization for CD3 cDNA content, mRNA expression for IL-12Rβ2, IFN-γ, and IL-2γ was determined by competitive RT-PCR. In each panel, the far left lane shows m.w. markers.
IL-12Rβ2 mRNA expression in human and murine T cell clones (9, 10). IL-10 and TGF-β decrease production of IL-12 (25, 26), and may therefore reduce IL-12R expression by T cells. To determine whether IL-10 and/or TGF-β contributed to reduced IL-12Rβ1 and IL-12Rβ2 expression by peripheral blood T cells in tuberculosis patients, neutralizing Abs to IL-10 and TGF-β were added to M. tuberculosis-stimulated PBMC from three tuberculosis patients. Addition of anti-IL-10 or anti-TGF-β increased IL-12Rβ1 expression 2- to 3-fold and increased IL-12Rβ2 expression 3- to 10-fold (Fig. 8).

In parallel with these changes and consistent with previously published results (22, 24), mean IFN-γ concentrations in supernatants of M. tuberculosis-stimulated PBMC from tuberculosis patients were increased from 296 ± 111 pg/ml to 1213 ± 64 pg/ml with addition of anti-IL-10, and to 1117 ± 65 pg/ml with addition of anti-TGF-β. These results suggest that IL-10 and TGF-β contribute to reduced expression of IL-12R and IFN-γ.

To directly demonstrate the effects of IL-10 and/or TGF-β on IL-12R expression by peripheral blood T cells in tuberculosis patients, we added these cytokines to M. tuberculosis-stimulated PBMC from healthy tuberculin reactors. In three experiments, IL-10 reduced IL-12Rβ2 expression by 76–85%, and TGF-β reduced IL-12Rβ2 expression by 48–84% (Fig. 9B). IL-10 and TGF-β also reduced IL-12Rβ1 expression (Fig. 9A), but the reduction was less striking than that observed for IL-12Rβ2. Parallel to the changes observed for IL-12Rβ2 protein, IL-10 and TGF-β reduced IL-12Rβ2 mRNA expression by 30–76% (data not shown). In addition, consistent with prior studies, mean IFN-γ concentrations in supernatants of M. tuberculosis-stimulated PBMC from four healthy tuberculin reactors were reduced from 2909 ± 489 pg/ml to 1666 ± 336 pg/ml with addition of IL-10, and to 1359 ± 469 pg/ml with addition of TGF-β.

![Figure 8](Image 63x435 to 286x733)

**FIGURE 8.** Effects of anti-IL-10 and anti-TGF-β on expression of IL-12Rβ1 (A) and IL-12Rβ2 (B) in M. tuberculosis-stimulated T cells from tuberculosis patients. PBMC from three tuberculosis patients were cultured in with M. tuberculosis, M. tuberculosis and anti-TGF-β, or M. tuberculosis and anti-IL-10. After 5 days, the percentages of IL-12Rβ1 + and IL-12Rβ2 + cells were measured by flow cytometry.

![Figure 9](Image 321x435 to 544x733)

**FIGURE 9.** Effects of TGF-β and IL-10 on expression of IL-12Rβ1 (A) and IL-12Rβ2 (B) in M. tuberculosis-stimulated PBMC from healthy tuberculin reactors. PBMC from three healthy tuberculin reactors were cultured with M. tuberculosis, M. tuberculosis and TGF-β, or M. tuberculosis and IL-10. After 5 days, the percentages of IL-12Rβ1 + and IL-12Rβ2 + cells were measured by flow cytometry.

Discussion

The results of this study show that expression of IL-12Rβ1 and IL-12Rβ2 parallels production of IFN-γ in human tuberculosis. In M. tuberculosis-stimulated peripheral blood T cells, IL-12Rβ1 and IL-12Rβ2 expression were reduced in tuberculosis patients with reduced IFN-γ production, compared with healthy tuberculin reactors with normal IFN-γ production. In contrast, at the site of disease in patients with tuberculous pleuritis and lymphadenitis, in which IFN-γ production is enhanced (17, 18), IL-12Rβ2 mRNA expression was increased in vivo. Addition of anti-IL-10 or anti-TGF-β to M. tuberculosis-stimulated peripheral blood T cells from tuberculosis patients enhanced expression of IL-12Rβ1 and IL-12Rβ2, and IFN-γ production. Conversely, addition of recombinant IL-10 or TGF-β to M. tuberculosis-stimulated PBMC from healthy tuberculin reactors decreased expression of IL-12Rβ1 and IL-12Rβ2, as well as IFN-γ production. These findings provide evidence that IL-12Rβ1 and IL-12Rβ2 expression correlate tightly with IFN-γ production in human disease from an intracellular pathogen, and suggest a relationship between expression of IL-12R and a protective Th1 response.

Host defenses against most intracellular pathogens are mediated by Th1 cells that produce IFN-γ, and severe manifestations of disease are generally associated with a reduced Th1 response. In extensive disease due to Mycobacterium leprae or Leishmania,
pressed Th1 responses probably result from enhanced production of the Th2 cytokines IL-4 and IL-10 (8, 27–30), which inhibit development of Th1 cells that produce IFN-γ (31). In diseases due to other intracellular pathogens such as Listeria monocytogenes, Salmonella, and M. tuberculosis, a reduced Th1 response is associated with severe disease, but there is no increase in the Th2 response (3, 17, 33). In these situations, a potential mechanism for diminished Th1 responses is through reduced effects of IL-12. The current findings extend these observations to a much larger population, demonstrating that decreased expression of IL-12Rβ1 and IL-12Rβ2 is characteristic of systemic T cells in tuberculosis patients. In these patients, M. tuberculosis-induced production of IFN-γ by T cells is depressed, but blood monocytes produce normal amounts of IL-12 and IL-10 (24, 39), suggesting that T cells have reduced responsiveness to IL-12. Addition of IL-12 to M. tuberculosis-stimulated PBMC markedly increases IFN-γ production (24), indicating that IL-12 responsiveness is partially restored by exogenous IL-12. These findings can now be explained by reduced IL-12Rβ1 and IL-12Rβ2 expression in T cells from tuberculosis patients, which diminishes responsiveness to IL-12. Addition of IL-12 up-regulates IL-12R expression, restores responsiveness to IL-12, and increases IFN-γ production (22, 24).

Several lines of evidence suggest that excess production of TGF-β may contribute to reduced IL-12R expression in tuberculosis. TGF-β can down-regulate IL-12Rβ2 expression and IL-12-mediated signal transduction in murine T cells, resulting in reduced IFN-γ production in response to IL-12 (40, 41). In addition, blood monocytes from tuberculosis patients produce high concentrations of TGF-β (22, 42), and the current study shows that anti-TGF-β enhances IL-12R expression and IFN-γ production by M. tuberculosis-stimulated T cells from tuberculosis patients. We therefore speculate that enhanced TGF-β production is the underlying abnormality that reduces IL-12R expression in tuberculosis. An alternative possibility is that Ag-reactive cells with increased IL-12R expression are recruited to the site of disease, reducing IL-12R expression in PBMC. Additional studies are needed to understand the mechanism underlying the depressed Th1 response in tuberculosis and in other diseases due to intracellular pathogens.

Acknowledgments

We thank Dr. Patrick Brennan for provision of M. tuberculosis Erdman.

References


