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Impairment of STAT Activation by IL-12 in a Patient with Atypical Mycobacterial and Staphylococcal Infections

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IL-12 plays a pivotal role in the stimulation of immune responses against intracellular infections. This role is manifested in the increased susceptibility to atypical mycobacterial and salmonella infections among individuals whose lymphocytes lack expression of IL-12Rβ1. Here, we report on a patient with Mycobacterium avium infection, recurrent Staphylococcus aureus sinusitis, and multiple adverse drug reactions whose T cells were unable to produce IFN-γ or proliferate in response to IL-12 despite the expression of wild-type IL-12Rβ1 and IL-12Rβ2. The defect in these functional responses to IL-12 was selective, as cytolytic activity induced by IL-12 was intact, and lymphocytes were responsive to stimulation by IL-2. An examination of cytokine signaling revealed that STAT4 and extracellular regulated kinase 1 (ERK1) activity by IL-12 was intact, whereas the activation of STAT1, -3, and -5 by IL-12 was lost. This impairment of STAT activation was specific for IL-12, as STAT activation by IL-2, IL-15, and IFN-γ was unaffected. These findings demonstrate that the activation of STAT4 alone is not sufficient for IL-12-induced IFN-γ production and proliferation and suggest that other STATs play a role in these responses to IL-12. While the etiology of the impaired IL-12 signaling in this patient has not yet been elucidated, the absence of mutations in IL-12Rβ1 or IL-12Rβ2 and the preservation of STAT4 activation raise the possibility that there may be a mutation in an as yet undiscovered component of the IL-12 signaling complex that is normally required for the recruitment and activation of STAT1, -3, and -5.


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Abbreviations used in this paper: ERK1, extracellular regulated kinase 1; JAK, Janus kinase.

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Mutations in cytokine signaling pathways in humans have provided important additional insights into the role that specific signaling proteins play in the activation of lymphocytes. The majority of mutations that have been reported involve cytokine receptor subunits, leading either to deficiencies in receptor expression or abnormalities in ligand binding (15, 16). For IL-12, mutations in the IL-12Rβ1 gene leading to loss of IL-12Rβ1 expression have been reported in children presenting with infections caused by atypical mycobacteria and salmonella (17, 18). These patients resembled those with IFN-γ receptor deficiencies, but their infections resulted from diminished IFN-γ production rather than a defect in IFN-γ signaling. Both T and NK cell responses to IL-12 in these patients were greatly diminished, thus demonstrating the importance of IL-12Rβ1 in lymphocyte IL-12 responsiveness.

Here we report on a young patient with an atypical mycobacterial infection, recurrent staphylococcal sinusitis, and multiple adverse drug reactions whose T cells were unable to proliferate or produce IFN-γ in response to IL-12 despite intact expression of wild-type IL-12Rβ1 and IL-12Rβ2. While IL-12 was able to stimulate the phosphorylation of STAT4 and ERK1 in her T cells, it was unable to induce the activation of STAT1, -3, or -5, all of which are activated by IL-12 in T cells from normal individuals. These findings provide new insights into how STAT activation affects functional responses to IL-12 and suggests that the defect in this patient involves an as yet undiscovered component of the IL-12 signaling complex distinct from IL-12Rβ1/IL-12Rβ2 and necessary for STAT1, STAT3, and STAT5 activation.

Materials and Methods

Case report

The patient is a 3-year-old Caucasian girl of Northern European extraction who presented at the age of 12 mo with fever and right cervical lymphadenitis. She responded partially to treatment with cephalexin, but 4 mo later had worsening cervical lymphadenitis with a strongly positive Mantoux test (30 × 30 mm erythema, 24 × 24 mm induration). One month later, she had a generalized tonic-clonic seizure, with a normal magnetic resonance imaging of the brain but an abnormal electroencephalogram, showing focal sharp waves in the left parieto-occipital region. She was started on phenytoin, but developed urticaria and a fine maculopapular rash and was changed to gabapentin. Because of her persistent cervical lymphadenitis and positive Mantoux test, she was begun empirically on rifampin and isoniazid, but these were discontinued soon afterward due to the development of a skin rash that was refractory to premedication and attempts at desensitization. She underwent surgical excision of multiple enlarged right cervical lymph nodes, which on histopathologic exam contained necrotizing granulomas. Cultures of the lymph nodes grew out Mycobacterium avium but were negative for Mycobacterium tuberculosis. A test for HIV was negative. The development of rashes to isoniazid, rifampin, and ciprofloxacin precluded the use of these antibiotics to treat the M. avium infection, and she was therefore started on an alternative regimen consisting of ampicillin plus amikacin.

An immune work-up revealed a normal complement of T, NK, and B cells with normal surface immunophenotype, normal neutrophil chemotaxis and nitro blue tetrazolium test, normal Ig levels (including IgM, IgA, IgE, IgG, and IgG subtypes) and isohemagglutinin titers, and normal proliferative response to mitogens such as PHA, Con A, and pokeweed mitogen. IL-12 production by PBMC in response to mitogens such as Con A and PHA was normal, but IFN-γ production in response to those same mitogens was diminished compared with that in normal controls.

Her family history (Fig. 1) was notable for adverse reactions (fever, hives, Arthus-like local reactions) to immunizations and antibiotics in all four of her siblings and death from cardiomyopathy in one of her sisters. The deceased sister also had a history of arthritis of unknown etiology and Staphylococcus aureus sepsis. Her two surviving sisters had recurrent episodes of S. aureus sinusitis. One of her surviving sisters also has nonatopic asthma and had a history of septic arthritis caused by Salmonella. Her mother had a history of breast cancer and nonatopic asthma, while her father has allergic rhinitis. A maternal aunt had a history of cervical lymphadenitis of unclear etiology (with strongly positive Mantoux test), numerous adverse reactions to medications, and recurrent sinusitis requiring multiple sinus surgeries.

Despite treatment with antibiotics the patient developed recurrent, extensive lymphadenitis in the right posterior cervical and submandibular

![Family pedigree for patient with M. avium infection.](http://www.jimmunol.org/)
regions, necessitating partial excision. The excised tissue contained necrotizing granulomas, but the culture for mycobacteria was negative. At that time, her development was noted to be delayed with regard to both growth and speech. An evaluation by a pediatric neurologist and psychiatrist suggested that this was a result of chronic illness, which included the recurrent lymphadenitis as well as recurrent episodes of *S. aureus* sinustis (treated once with surgical drainage) responsive to amoxicillin/clavulanate. Given the low IFN-γ production by PBMC in response to mitogens and persistent swelling/drainage from the right cervical region, she was begun on IFN-γ (1.5 × 10^5 U/mg administered s.c. every other day). Over the next few weeks her surgical wound stopped draining and healed, and her lymphadenitis resolved. She has continued on chronic IFN-γ therapy, and over the past year she has had no new sinuses infections or lymphadenitis. Both her growth and speech development have normalized, and she no longer exhibits adverse reactions to various food Ags. In addition, she has tolerated her childhood immunizations without adverse reactions and has had normal Ab responses to diphertheria toxin, ProHib, and Pneumovax.

**Abs and cytokines**

The surface expression of IL-12Rβ1 was detected using the previously described 12Rβ3F12 Ab (19). A mAb recognizing IL-12Rβ2 (20) was provided by Dr. Jerome Ritz. The Ab to the α-chain (p55) of the IL-2R was purchased from PharMingen (San Diego, CA). An anti-IL-12 Ab (C88.6.2) that is able to bind IL-12 complexed to its receptor was provided by the Bioanalytical Sciences Department of Genetics Institute (Cambridge, MA). MOPC-21 (Sigma, St. Louis, MO) served as a mouse IgG1 negative control Ab. Biotin-conjugated goat anti-mouse IgG1 and streptavidin-PE were purchased from Southern Biotechnologies (Birmingham, AL). The phospho-STAT1Ab recognizes the tyrosine-phosphorylated forms of STAT1α and STAT1β and cross-reacts with the tyrosine-phosphorylated form of STAT5 (8). The phospho-STAT3 and phospho-STAT3 Abs specifically recognize the tyrosine-phosphorylated forms of STAT5 and STAT3, respectively (8, 21). The Abs to STAT1, STAT3, and STAT4 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phosphoERK1/ERK2 and anti-ERK1/ERK2 were purchased from New England Biolabs (Beverly, MA). The anti-phosphotyrosine Ab (4G10) was a gift from Dr. Thomas Roberts (Dana-Farber Cancer Institute, Boston, MA). HRP-conjugated rabbit anti-mouse and goat anti-rabbit Abs were purchased from Calbiochem (San Diego, CA).

Purified human IL-12 (sp. act., 4.7 × 10^8 U/mg) was provided by Genetics Institute, and IL-2 (sp. act., 18 × 10^9 U/mg) was supplied by Chiron (Emeryville, CA). IL-15 (sp. act., >2 × 10^8 U/mg) and IL-18 were purchased from Endogen (Cambridge, MA). IFN-γ (sp. act., 1 × 10^7 U/mg) was obtained from R&D Systems (Minneapolis, MN), and IFN-α2b (sp. act., 2 × 10^8 U/mg) was provided by Schering-Plough (Madison, NJ).

**Isolation and activation of PBMC**

Heparinized blood samples were obtained from the patient and from healthy adult volunteers. The blood draws and immunologic studies involving cells obtained from the patient were performed as part of protocol 9606, approved by the institutional review board at the University of Minnesota. PBMC were isolated from blood samples through density gradient centrifugation using Histopaque-1077 (Sigma). For T cell activation studies, PBMC were cultured for 96 h in RPMI 1640 containing 15% heat-inactivated FBS and 1% penicillin/streptomycin. PBMC were split into two aliquots. One was cultured alone for 72 h, and the other was cultured with 10 U/ml of IL-2. After 96 h of activation, cells were routinely >95% CD3 and CD56.

**Proliferation assays and measurement of IFN-γ production**

Following activation with PHA plus IL-2 for 96 h, T cells were incubated in complete RPMI-U-bottom plates at 3 × 10^6 cells/well with medium alone or the indicated concentration of cytokine at 37°C. Proliferation assays consisted of a 96-h incubation, with 1 μCi of [3H]thymidine (DuPont-NEN, Boston, MA) added 8 h before harvesting. For IFN-γ assays, supernatants were harvested after a 72-h incubation, and the IFN-γ concentration was assayed using an ELISA kit (Endogen, Cambridge, MA).

**Cytotoxicity assays**

A lactate dehydrogenase release assay (CytoTox 96 Non-Radioactive Cytotoxicity Assay, Promega, Madison WI) was used for measuring cytotoxicity. Whole PBMC were first incubated overnight in U-bottom 96-well plates with medium alone, 1 μM IL-2, 100 U/ml IL-2, or IL-2 plus IL-18 at 37°C. K562 cells were then added to the PBMC at a 10:1 E:T cell ratio. PBMC were incubated with the K562 targets for 4 h at 37°C, and the supernatants were harvested and assayed for lactate dehydrogenase release.

**Immunoprecipitations and Western blots**

T cells activated with PHA and IL-2 for 72 h were washed and recultured for an additional 18 h in starvation medium consisting of RPMI 1640 and 2.5% FCS before cytokine stimulation. Activated T cells or unactivated PBMC were stimulated for 20 min at 37°C with medium alone, 1 nM rhIL-12, 10 U/ml IL-2, 10 ng/ml IL-15, 5 nM IL-18, 10 U/ml IFN-α2b, or 100 U/ml IFN-γ. After stimulation, cells were washed once with ice-cold PBS and then lysed on ice for 20 min in lysis buffer containing 1% Nonidet P-40, 50 mMol/L Tris (pH 8.0), 150 mMol/L NaCl, 2 mMol/L EDTA, 2 μg/ml aprotinin, 100 μg/ml PMSF, 1 mMol/L sodium orthovanadate, and 1 mMol/L NaF. Aliquots of whole cell lysates were mixed with reducing sample buffer and boiled, and proteins were resolved on a polyacrylamide gel. For immunoprecipitations, a STAT4 Ab was added to the lysates, which were then incubated overnight at 4°C. Ab-protein complexes were subsequently immunoprecipitated from the lysates by adding protein A-Sepharose beads (Pharmacia Biotech, Uppsala, Sweden) and incubating with rotation at 4°C for 4 h. The beads were washed twice with ice-cold PBS and then boiled in reducing sample buffer, and precipitated proteins were resolved on a polyacrylamide gel.

For Western blots, fractionated proteins were transferred from polyacrylamide gels to nitrocellulose membranes by electrophobbing, and membranes were then blocked for 30 min in TBS containing 0.1% Tween 20 (Bio-Rad, Hercules, CA) and 5% BSA or 5% nonfat dried milk. Membranes were then incubated with dilutions of the indicated Abs for 1 h at room temperature, washed with TBST, incubated with either HRP-conjugated rabbit anti-mouse or goat anti-rabbit Abs for 1 h, washed again, and developed using enhanced chemiluminescence (Amersham, Aylesbury, U.K.). When reprobed, membranes were first stripped by incubating in a solution containing 2% SDS, 100 mMol/L 2-ME, and 62.5 mMol/L Tris-HCl, pH 6.7, for 30 min at 65°C.

**Immunofluorescence analysis**

Cytokine receptor staining was performed with a three-step method using a primary unconjugated Ab specific for the indicated receptor subunit, a biotin-conjugated anti-mouse IgG1 secondary Ab, and streptavidin-PE. An irrelevant unconjugated IgG1 Ab was used as the negative control in these experiments.

To assess IL-12 binding (22), T cells activated with PHA and IL-2 for 72 h were incubated on ice with either medium alone or 10 nM rhIL-12 for 1 h. Cells were then washed, incubated with an anti-IL-12 Ab, washed again, and incubated with a PE-conjugated goat anti-mouse IgG1 Ab. After staining, cells were fixed with 1% formaldehyde and analyzed by flow cytometry.

**Sequencing of IL-12R subunits**

**IL-12Rβ1.** Extraction of total RNA from 20 × 10^6 T cells activated for 5 days with PHA and IL-2, cDNA synthesis, and PCR were performed as previously described (23). Primers for amplification of the IL-12Rβ1 cDNA coding region were 5′-TGAACCTCGGAGGTGGCA-3′ (sense) and 5′-TCCGGCGGACTACTACCACT-3′ (antisense). Sequencing was performed with an ABI Prism dRhodamine Terminator kit and analyzed with an ABI Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). A series of nested primers was used for sequencing.

**IL-12Rβ2.** cDNA was isolated by the TRizol method from 20 × 10^6 T cells activated for 5 days with PHA and IL-2. The purified RNA was reverse transcribed using oligo(dT)12 and mouse Moloney murine leukemia virus reverse transcriptase. Ten percent of the RT mix was used to PCR amplify the cytoplasmic region of IL-12Rβ2 using a reverse oligonucleotide (5′-ATGCGCCGCTGGCCGGCGC-3′; positions 3291–3267), which hybridized to the 3′ untranslated region, and a forward oligonucleotide (5′-GGTGCGCCATTTCTCAACGGCATTAC-3′; positions 2551–2575), which hybridized to the transmembrane region of the same RT mix. The PCR primers were used to amplify the extracellular region using a reverse oligonucleotide (5′-AAGTAATGCGCTGGCAGAAGTAGGBCCACC-3′; positions 2578–2551), which hybridized to the 3′ transmembrane region, and a forward oligonucleotide (5′-CGTGGAGGATCCGGGCTGCTGGC-3′; positions 601–626), which hybridized to the 5′ untranslated region. The resulting PCR product (1661 bp, for IL-2, or 1681 bp, for IL-12) was sequenced (Tutogen Biotech, Medical Research Facility, Medford, MA) using the oligonucleotides above and the additional sequential overlapping oligonucleotides to complete the sequence shown in Table I.
Results

IL-12-induced proliferation and IFN-γ production are impaired but cytolytic activity is preserved in lymphocytes from the patient

To determine whether the diminished ability of PHA to stimulate IFN-γ production from the patient’s PBMC was indicative of an IL-12-based immunodeficiency, we examined the response of her PHA-activated T cells to IL-12. As shown in Fig. 2 her T cells failed to proliferate or produce IFN-γ in response to concentrations of IL-12 ranging from 10–1000 pM. To determine whether this represented a global abnormality in functional responses to cytokines or was specific for IL-12, we tested the response of her T cells to IL-2. While IL-2-induced proliferation was intact (Fig. 2A), IFN-γ production in response to IL-2 was greatly diminished (Fig. 2B). A similar decrease in IL-2-induced IFN-γ production was observed when activated T cells from a healthy control were stimulated with IL-2 in the presence of a neutralizing IL-12 Ab (data not shown).

To determine whether IL-12-induced cytolytic activity was affected along with proliferation and IFN-γ production, we examined the ability of IL-12 and IL-2 to stimulate cytotoxicity against the NK-sensitive cell line K562. Although the baseline killing of K562 was less with PBMC from the patient compared with control PBMC, the augmentation of cytotoxicity by IL-12, IL-2, and IL-12 plus IL-2 was similar for both patient and controls (Fig. 2C).

Diminished IL-12 responsiveness is not due to alterations in IL-12R subunit expression or primary structure

As the functional response of lymphocytes to IL-12 is dependent on the expression of high affinity IL-12R, we examined whether the selective loss of IL-12-induced proliferation and IFN-γ production in our patient was due to a deficiency in the expression of the β1 and/or β2 subunits of the high affinity IL-12R. Cell surface staining of PHA-activated T cells with Abs specific for IL-12Rβ1 and IL-12Rβ2 revealed that T cells from the patient and control T cells were comparable with respect to IL-12R subunit expression (Fig. 3A). A similar degree of IL-12 binding to activated T cells was also detected (Fig. 3B).

Table I. Sequencing of IL-12Rβ2

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<tr>
<th>Position</th>
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<tr>
<td>2581–2601</td>
<td>GCAAAAGGTGTTTGTCCTCCT (forward)</td>
</tr>
<tr>
<td>2861–2881</td>
<td>AAAGACATGATGCACTGCC (forward)</td>
</tr>
<tr>
<td>3071–3051</td>
<td>AGGGGAGGCATCTAGTGG (reverse)</td>
</tr>
<tr>
<td>2786–2765</td>
<td>GGCTACCTGATAGACAGCT (reverse)</td>
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<tr>
<td>1037–1057</td>
<td>ATACAGAAGGAGAACAGG (forward)</td>
</tr>
<tr>
<td>1451–1460</td>
<td>AATGTACAAAGGCCAAAG (forward)</td>
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<tr>
<td>2292–2273</td>
<td>CCCATTGCTTCTGGACCTG (reverse)</td>
</tr>
<tr>
<td>1950–1932</td>
<td>ATGTTGTCCATGCGCCTC (reverse)</td>
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FIGURE 2. Functional responses to IL-12 and IL-2 in lymphocytes from the patient. PBMC isolated from either the patient or a healthy control were first activated with PHA and IL-2 for 96 h and then stimulated with the indicated concentrations of IL-12 or IL-2 for an additional 72–96 h before measuring proliferation (A) and IFN-γ production (B). Cytolytic activity against the K562 cell line (C) was determined following overnight stimulation of freshly isolated PBMC from either patient or control with IL-12, IL-2, or IL-12 plus IL-2. Results are representative of two separate experiments using cells from two different controls.
To exclude the possibility of a mutation in either the $\beta_1$ or $\beta_2$ receptor that could affect the function of the IL-12R without modulating its expression, we sequenced the $\beta_1$- and $\beta_2$-coding region cDNAs derived from the patient’s activated T cells. Both were determined to be wild type (data not shown).

IL-12 activates STAT4 in T cells from the patient but fails to activate STAT1, STAT3, or STAT5

As data from STAT4 knockout mice have shown that STAT4 is necessary for lymphocyte IL-12 responsiveness, we examined whether the inactivity of the patient’s activated T cells to proliferate or produce IFN-$\gamma$ in response to IL-12 was due to a loss of STAT4 activation by IL-12. As shown in Fig. 4, IL-12 was able to stimulate both the tyrosine and serine phosphorylation (24) of STAT4 in activated T cells from the patient and controls. However, although IL-12 could activate STAT1, STAT3, and STAT5 in T cells from controls (Fig. 5A), it was unable to activate STAT5 in the patient, and only weakly augmented the activation of STAT1 and STAT3 over the baseline levels (Fig. 5B). This impairment of STAT1, STAT3, and STAT5 activation by IL-12 was not due to diminished expression of these STATs (Fig. 5B).

To determine whether the activation of these same STATs by other cytokines was also affected, we examined the activation of STAT1, -3, and -5 by IL-2, IL-15, and IFN-$\alpha$. As shown in Fig. 5B, STAT activation by these cytokines was preserved, showing that the JAK/STAT signaling pathways for IL-2, IL-15, and IFN-$\alpha$ were intact in the patient. In addition, STAT1 activation by IFN-$\gamma$ was unaffected (Fig. 5C).

ERK activation by IL-12 is intact in T cells from the patient

While ERK1 activation by IL-12 has been observed in mitogen-activated T cells, the role that ERK1 plays in mediating IL-12 functional responses remains undefined. In activated T cells from the patient, ERK1 and ERK2 were expressed at levels comparable to those in controls (Fig. 6). While the basal amount of ERK1 phosphorylation was greater in activated T cells from controls compared with the patient, the increases in ERK1 phosphorylation stimulated by IL-2 and, to a lesser degree, IL-12 were similar in both (Fig. 6).

Discussion

In this report we have characterized an immunodeficiency that is caused by a defect in IL-12 STAT signaling. Although the atypical mycobacterial infection in our patient resembles those reported in young patients with mutations in the IL-12R$\beta_1$ subunit (17, 18) or in the IL-12 p40 gene (25), this case is unique in that IL-12 expression was normal but lymphocyte IL-12 responsiveness was impaired despite the normal expression of wild-type IL-12R$\beta_1$ and IL-12R$\beta_2$ subunits. In addition, while defects in IL-12 or IL-12R$\beta_1$ expression were associated solely with intracellular infections by poorly virulent mycobacteria and salmonella, the phenotype of our patient also included susceptibility to staphylococcal infections as well as multiple adverse food and drug reactions.

As was observed with lymphocytes from patients with IL-12R$\beta_1$ mutations, the ability of IL-12 to stimulate lymphocyte IFN-$\gamma$ production and proliferation was completely abolished in our patient. However, while IL-12R$\beta_1$ mutations also affected the ability of IL-12 to augment lymphocyte cytolytic activity (17), this IL-12 function was preserved in our patient with intact IL-12R$\beta_1$. 

**FIGURE 3.** IL-12R expression and IL-12 binding in the patient vs normal control. A, PBMC from the patient or control were activated with PHA and IL-2 and then stained using Abs specific for the indicated subunits of the IL-2 or IL-12 receptor. B, Activated T cells from patient or control were stained with a nonneutralizing anti-IL-12 Ab before and after incubation with IL-12. Results are representative of two separate experiments using cells from two different controls.

**FIGURE 4.** Analysis of STAT4 activation by IL-12. PBMC isolated from patient or control were activated with PHA and IL-2 for 72 h, rested overnight, and then stimulated for 20 min with either IL-2 or IL-12. STAT4 was immunoprecipitated (I.P.) from cells as described in Materials and Methods, and the extent of tyrosine phosphorylation (p-STAT4) was assessed through Western blotting with a phosphotyrosine (\(\alpha\)-p-tyr) Ab (A). Serine phosphorylation of STAT4 (ps-STAT4), represented by a shift in the STAT4 band (24), was determined through Western blotting with a STAT4 Ab (B). Results are representative of two separate experiments using cells from the patient and from two different controls.
and IL-12Rβ2 expression. This finding suggests that the production of IFN-γ by T and NK cells and the resulting activation of macrophages is more critical to the eradication of intracellular organisms such as salmonella and mycobacteria than lymphocyte cytolytic activity. The therapeutic effect of chronic IFN-γ administration in our patient further underscores the role of IFN-γ in the control of mycobacterial infections (26). In addition, the prevention of further episodes of staphylococcal sinusitis by IFN-γ therapy suggests that IFN-γ is an important component of immunity directed against deep-seated infections by bacteria such as S. aureus.

IL-2-induced T cell proliferation and lymphocyte cytolytic activity were normal in our patient. In contrast, IL-2-induced IFN-γ production was impaired, suggesting a possible defect in the regulatory sequences within the IFN-γ gene. However, her PBMC were able to produce IFN-γ in response to PHA, albeit at a diminished level compared with controls. Furthermore, a similar, selective defect in IL-2-induced IFN-γ production was demonstrated for patients with IL-12Rβ1 mutations (18). This phenomenon probably reflects the important role that IL-12 costimulation plays in IFN-γ induction by IL-2 (27, 28) and suggests that endogenous IL-12 contributes significantly to the ability of IL-2 to induce IFN-γ production in vitro using activated PBMC. This is further supported by our observation that a neutralizing IL-12 Ab impaired IL-2-induced IFN-γ production in assays using activated PBMC from controls. Therefore, it is not necessary to invoke a defect in the IFN-γ gene per se to explain the failure of both IL-12 and IL-2 to induce its expression. Rather, the decrease in IFN-γ production by IL-2 is more likely a reflection of the defect in IL-12 responsiveness.

As STAT4 activation by IL-12 has been shown to be dependent on the expression of both IL-12Rβ1 and IL-12Rβ2 (14), it was not surprising to observe that IL-12 was able to stimulate both the tyrosine and serine phosphorylation of STAT4 in activated T cells from our patient. This finding provides further evidence that her lymphocytes expressed functional high affinity IL-12R that could bind IL-12. However, what was unexpected was that T cell IFN-γ production and proliferation were not induced by IL-12, even though IL-12 was able to activate STAT4 in T cells. It is unlikely that the function of STAT4 was impaired despite the ability of IL-12 to induce its phosphorylation, for we also found that STAT4 DNA binding in response to IL-12 was intact in the patient and have established that the STAT4 cDNA sequence was wild type (data not shown).

Importantly, the loss of T cell functional responses to IL-12 was associated with the inability of IL-12 to activate STAT1, -3 and -5. Therefore, while murine models have shown that STAT4 is necessary for lymphocyte IL-12 responsiveness (13), our data indicate that STAT4 activation alone may be sufficient for the induction of cytolytic activity by IL-12 but may not be sufficient for the induction of IFN-γ production or proliferation. Rather, the activation of IFN-γ by IL-12 is dependent on STAT4 and STAT6 (13). This is consistent with our previous observation that STAT6 activation by IL-2 was normal in our patient, supporting the view that STAT6 activation by IL-2 is independent of STAT4 activation by IL-2.

**FIGURE 5.** IL-12-induced activation of STAT1, -3, and -5 is impaired in the patient. PBMC isolated from a control (A) or from the patient (B) were activated for 72 h with PHA plus IL-2, rested overnight, and then stimulated for 20 min with the indicated cytokines. Western blots were performed on whole cell lysates using Abs specific either for the indicated STATs or for the tyrosine-phosphorylated forms of the indicated STATs (p-STAT). The anti-phospho-STAT1 Ab (upper panel, A and B) recognizes the tyrosine-phosphorylated forms of STAT1α, STAT1β, and STAT5. The results shown are representative of three separate experiments using cells from the patient and from three healthy controls. C, STAT1 activation was examined in unstimulated and IFN-γ-stimulated PBMC from the patient. Results are representative of two separate experiments.

**FIGURE 6.** ERK1 activation by IL-12 is similar in patient and controls. Activated T cells from the patient or a control were stimulated with either IL-12 or IL-2, and Western blots were performed on whole cell lysates using Abs specific for either ERK1/2 (bottom) or the activated, phosphorylated forms of ERK1/2 (top). Results are representative of two separate experiments. α-p, anti-phospho.
STAT1, STAT3, and/or STAT5 appears to be required in addition to STAT4 activation for IL-12 to elicit the full array of responses to IL-12. As weak activation of ERK1 by IL-12 was observed in T cells from both the patient and controls, it is doubtful that it plays a significant role in IL-12-induced proliferation and IFN-γ production. It may, however, play a role along with STAT4 in IL-12-induced cytolytic activity. Our observation that the activation of STAT1, -3, and -ERK1 by IL-2 was preserved in our patient is further evidence that a selective defect in IL-12 signaling was responsible for the observed immunodeficiency.

Although it appears likely that the loss of activation of STAT1, -3, and -5 by IL-12 was responsible for the impairment of lymphocyte IL-12 responsiveness, we have not yet elucidated the cause of this signaling defect. The history of cervical lymphadenitis and/or recurrent staphylococcal sinusitis in multiple family members raises the possibility that a heritable abnormality in the IL-12 signaling pathway may underlie the clinical phenotype of the patient. While these notable clinical features did not appear to exhibit a Mendelian mode of inheritance, an examination of T cell IL-12 responsiveness and IL-12-induced STAT activation in family members should further aid in determining whether a single genetic mutation may be operative in the susceptibility to infection.

As the cytoplasmic regions of IL-12Rβ1 and IL-12Rβ2 were intact in the patient, the inability to activate select STATs is unlikely to have been caused by an abnormality in STAT recruitment to those receptor subunits. Furthermore, since the JAK/STAT family of kinases is likely to have been caused by an abnormality in STAT recruitment intact in the patient, the inability to activate select STATs is unexplained. The functional synergy between IL-12 and IFN-γ protein kinase and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunology 2:171.


