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8. Patient 1 was reported in a series of patients with idiopathic disseminated BCG infection [patient 9 in (*3*)]. She also had *Salmonella enteritidis* infection, and when reviewed at 19 years of age, she remained well off therapy. Four siblings were vaccinated with BCG with no adverse effects. Patient 2 suffered from disseminated BCG infection [O. Jeppsson, B. Petrini, J. Andersson, N. Heurlin, G. Malm, *Lancet* **ii**, 570 (1988)]. When reviewed at 11 years of age, she remained well off therapy. Three siblings were vaccinated with live BCG with no adverse reactions, and another sibling, also vaccinated, died of fever of unkown cause at 1 year of age. Patient 3 had not been vaccinated with BCG and suffered from *S. enteritidis* infection at 11 and 20 years of age and *Mycobacterium avium* infection at 24 years of age (*1*). Mycobacterial infection improved only after IFN-γ therapy was added to antibiotics, and when reviewed at 29 years of age, he remained well off all therapy for 3 years. His brother, patient 4, died of disseminated *M. avium* infection at 8 years of age. Two sisters, aged 17 and 24 years, are well.
9. Immunological investigations included (i) normal serum complements; (ii) increased serum immunoglobulin M (IgM) (2 to 4 g/liter), IgA (2 to 5 g/liter), IgG (10 to 30 g/liter), and IgE (20 to 50 kUI/ml); (iii) protective serum antibody titers to *Clostridium tetani* toxoid and poliovirus after immunization; (iv) normal blood NK, B, and T cell numbers; and (v) normal proliferation of T cells in response to mitogens (phorbol 12-myristate 13-acetate–ionomycin and PHA) and recall antigens (tuberculin, poliovirus, and *C. tetani* toxoid). Mutations in IFN-γR1 and IFN-γR1–associated molecules were excluded by normal cellular responses to IFN-γ in vitro (*4*). Mutations in IFN-γ and IL-12 were unlikely, as assessed by cytokine detection in the supernatant of cultured activated peripheral blood cells.
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13. Extraction of total RNA from PBMCs or Epstein-Barr virus–transformed B cells, cDNA synthesis, and the polymerase chain reaction (PCR) were performed as described (*4, 6*). Primers for amplification of the IL-12Rβ1 cDNA coding region were 5′-TGAACCTCG-CAGGTGGCAGA-3′ (sense) and 5′-TCGGGC-GAGTCACTCACCCT-3′ (antisense) (*12*). Sequencing was done with an Abi Prism dRhodamine Terminator kit and analyzed with an Abi Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems). A series of nested primers were used for sequencing (available on request).
14. Extraction of genomic DNA was done from blood cells (*4, 6*). A series of primers for PCR and sequencing, based on the published sequence of the cDNA, were synthesized for amplification of the genomic mutation (available on request). For the analysis of intrafamilial segregation of the mutation, a genomic PCR surrounding nucleotide 913 was digested with Mbo II (Boehringer) and run on an agarose gel.
15. Flow cytometry analysis of IL-12Rβ1 cell surface expression on activated T cells was done after activation of fresh PBMCs or cultured *Herpesvirus saimiri*–transformed T cells [E. Meinl, R. Hohlfeld, H. Wekerle, B. Fleckenstein, *Immunol. Today* **16**, 55 (1995)] by PHA (20 μg/ml; Difco) in RPMI 1640 medium supplemented with 10% human AB serum for 72 hours. Mouse IgG1 mAbs 12Rb.44 or 12Rb.3F12 [J. A. Gollob, H. Kawasaki, J. Ritz, *Eur. J. Immunol.* **27**, 647 (1997)] were revealed by biotinylated goat antibody to mouse IgG1 (Rockland) in combination with streptavidin-phycoerythrin (Tebu, France).
16. PBMCs were purified by Ficoll-Hypaque density gradient separation and cultured in RPMI 1640 supplemented with 2% heat-inactivated fetal bovine serum. As a test of NK activity, PBMCs were incubated with K562 cells [F. Le Deist *et al.*, *J. Immunol.* **138**, 423 (1987)], with or without recombinant IL-12 (40 ng/ml); supernatants were harvested at 4 hours for ^{51}Cr release quantification and at 18 hours for IFN-γ quantification by enzyme-linked immunosorbent assay (ELISA; R&D Systems).
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18. PBMCs were stimulated with PHA (1:700 dilution; Difco) or with tuberculin (5 μg/ml; Statens Serum Institute, Copenhagen). IFN-γ was quantified in the supernatant after 48 hours by ELISA, and cell proliferation was measured by incorporation of radiolabeled nucleotides after 3 days for PHA and after 5 days for tuberculin.
19. DTH to tuberculin-purified protein derivative (PPD) was assessed by intradermal inoculation of 10 IU of PPD and measurement of skin induration after 48 to 72 hours. DTH was found to be positive (induration >10 mm) in patients 1 and 2 after BCG vaccination.
20. Materials analyzed from BCG-infected children included (i) enlarged lymph nodes and liver taken 3 and 13 months after BCG inoculation in patient 1 (before any antibiotic therapy was commenced); (ii) enlarged lymph nodes of four immunocompetent children with BCG-itis; and (iii) enlarged lymph nodes of four children with disseminated BCG infection and complete (n = 3) or partial (n = 1) IFN-γR1 deficiency. Slides were stained with hematoxylin-eosin and Ziehl-Neelsen stain. Immunochemistry was done with primary antibodies specific for CD3ε (rabbit antibody to human CD3; Dako, Copenhagen), CD8 (C8/144B, Dako), CD4 (MT310, Dako), CD45RO (UCHL1, Dako), and GMP-17 (TIA-1; Coulter, Hialeah, FL). GMP-17 is a protein associated with cytotoxin granules of CD8 T cells and NK cells [A. Anderson *et al.*, *J. Immunol.* **144**, 574 (1990); Q. G. Medley *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 685 (1996)].
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32. We thank J. Peake for critical reading, P. Brousset for the CD4 staining, and M. Forveille for technical assistance. J.-L.C. thanks B. Malissen for insightful advice. Supported by Fondation Marcel Mérieux (F.A.), Glaxo-Wellcome Action TB Programme (D.L.), Ligue Nationale contre le Cancer (E.J.), Association Recherche et Partage (S.L.), INSERM (R.D.), Immuno France, and grants from INSERM, Association Française contre le Myopathies, Programme Hospitalier de Recherche Clinique, Medical Research Council, and The West Midlands NHS Regional Research Funds.

29 December 1997; accepted 31 March 1998

Severe Mycobacterial and *Salmonella* Infections in Interleukin-12 Receptor-Deficient Patients

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Interleukin-12 (IL-12) is a cytokine that promotes cell-mediated immunity to intracellular pathogens by inducing type 1 helper T cell (T_H1) responses and interferon-γ (IFN-γ) production. IL-12 binds to high-affinity β1/β2 heterodimeric IL-12 receptor (IL-12R) complexes on T cell and natural killer cells. Three unrelated individuals with severe, idiopathic mycobacterial and *Salmonella* infections were found to lack IL-12Rβ1 chain expression. Their cells were deficient in IL-12R signaling and IFN-γ production, and their remaining T cell responses were independent of endogenous IL-12. IL-12Rβ1 sequence analysis revealed genetic mutations that resulted in premature stop codons in the extracellular domain. The lack of IL-12Rβ1 expression results in a human immunodeficiency and shows the essential role of IL-12 in resistance to infections due to intracellular bacteria.

IL-12 is a heterodimeric cytokine that consists of two disulfide-linked subunits, p40 and p35, and is produced by activated antigen presenting cells (dendritic cells, macrophages), particularly upon infection with intracellular microbes (*1, 2*). IL-12 promotes the development of T_H1 responses and is a powerful inducer of IFN-γ production by T

cells and natural killer (NK) cells (*1, 2*). The receptor for IL-12 is composed of two distinct subunits, β1 and β2, that assemble to form a high-affinity IL-12R complex expressed on T cells and NK cells (*3–6*). IL-12 and IFN-γ appear to be both essential for the development of protective cell-mediated immunity to tuberculous mycobacterial pathogens in mice (*7*) and in humans (*8*).

Idiopathic disseminating mycobacterial infections due to nontuberculous species (*Mycobacterium fortuitum, Mycobacterium avium, Mycobacterium chelonei, Mycobacterium smegmatis*) or *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) have been described in patients without previously recognized immunodeficiencies (*9–13*). Peripheral blood mononuclear cells (PBMCs) of some patients were deficient in IFN-γ production upon mitogenic polyclonal stimulation (*10, 14*). In several cases genetic analyses identified inactivating mutations in the IFN-γR1 gene, resulting in complete (*10, 12–14*) or partial (*15*) deficiencies in IFN-γR expression or function. In another family with similar disseminating *M. avium* infections, IL-12 production was reported to be deficient in affected members, but the underlying mechanism was not elucidated (*16*).

We have examined type-1 cytokine and T_H1 responses in three unrelated individuals with recurrent, severe mycobacterial and *Salmonella* infections. Patient 1 is a 26-year-old female who developed a severe *Salmonella paratyphi* sepsis at the age of 3 years, which was complicated by abdominal abscesses, and at the age of 22 years presented with a *M. avium* sepsis with extensive mediastinal lymphadenopathy. Patient 2, a 19-year-old female, presented with recurrent systemic *M. avium intracellulare* infections at ages 4, 13, and 17 years and with severe systemic *Salmonella* type B infections at ages 4, 7, and 14 years. Patient 3 is a 3-year-old female who developed progressive *M. bovis* BCG infection after vaccination at the age of 1 year, followed by severe and nearly fatal *S. typhimurium* sepsis at the age of 2 years. Upon histological examination, the BCG lesion of patient 3 contained well-organized granulomatous infiltrates (*17*). None of the three patients had any recognized immunodeficiencies or alterations in expression of T, B, NK, or macrophage cell surface markers (*18*). All three patients could be treated effectively with antibiotic therapy (*18*).

To investigate the nature of these immunodeficiencies, we stimulated PBMCs derived from patients and healthy controls ($n = 4$) with mitogenic combinations of T cell-specific monoclonal antibodies (mAbs) to CD2 and CD28 (*19, 20*), IL-2, or the lectin phytohemagglutinin (PHA). Although PBMCs from patients as well as controls proliferated to all these stimuli (Figs. 1A and 2B), patients' PBMCs were deficient in IFN-γ production [Figs. 1B and 2, C and E (*21*); 85 to 99% reduction compared with controls]. Similar deficiencies in IFN-γ production were observed in response to the pathogens *M. avium*, *M. tuberculosis*, and *S. paratyphi* (*21*). These results were consistently reproducible in multiple experiments.

To exclude the possibility that defective IFN-γ production in the patients was associated with a lack of IFN-γR expression (*10, 13*), we determined cell surface expression of IFN-γR (CD119) and responsiveness to exogenous recombinant IFN-γ (rIFN-γ). CD119 expression on freshly isolated $CD14^+$ monocytes from patients was similar to that of controls (*21*), and incubation of patient and control cells with rIFN-γ enhanced tumor necrosis factor-α (TNF-α) production triggered by lipopolysaccharide (LPS) (*22*). rIFN-γ also synergized with *M. avium* in inducing the production of IL-12 p40 and IL-12 p70 (Fig. 1C). LPS alone also induced IL-12 p70 release in patients. Thus, all three patients were deficient in IFN-γ production, yet they displayed normal IL-12 p70 production, IFN-γR expression, and IFN-γR function.

Therefore, we examined IL-12R expression and function. We measured IL-12Rβ1 expression on PHA-activated T cells from patients and controls by using the IL-12Rβ1-specific mAb 2.4E6 (*23*). Cells from patients did not express detectable IL-12Rβ1 cell surface molecules, whereas expression of the T cell activation marker CD25 was similar

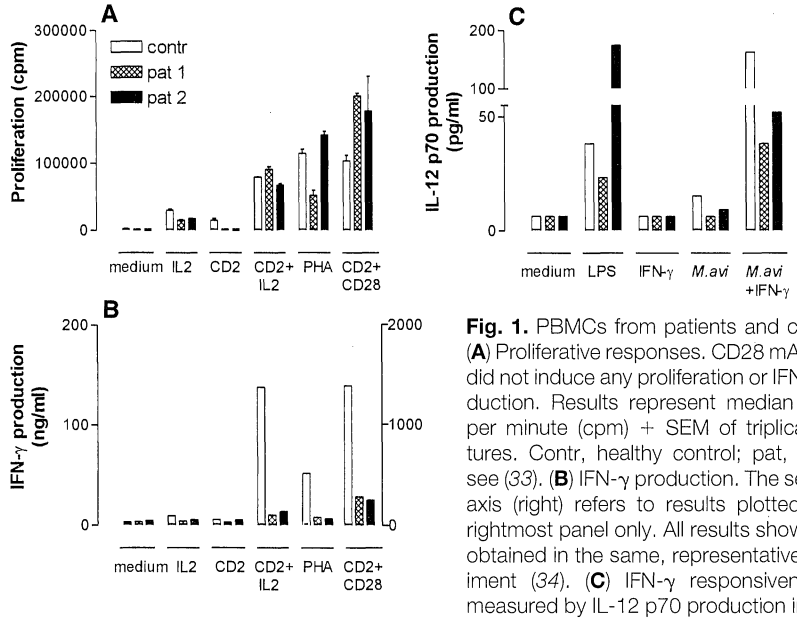

Fig. 1. PBMCs from patients and controls. (**A**) Proliferative responses. CD28 mAb alone did not induce any proliferation or IFN-γ production. Results represent median counts per minute (cpm) + SEM of triplicate cultures. Contr, healthy control; pat, patient; see (*33*). (**B**) IFN-γ production. The second *y* axis (right) refers to results plotted in the rightmost panel only. All results shown were obtained in the same, representative experiment (*34*). (**C**) IFN-γ responsiveness as measured by IL-12 p70 production in whole blood cultures. Similar results were obtained in IL-12p40 ELISAs (*35*).

Table 1. Patients with genetic lack of IL-12R expression. Mutations are indicated according to (*37*). X is a stop codon; Q is glutamine. Mutations shown are homozygous in the patients only; all parents were heterozygous (*30*). Each of the three mutations leads to a premature stop codon in the extracellular domain-encoding region, resulting in lack of membrane expression. See text for discussion.

Patient	Origin	Consanguinity	Infection	Mutation
1	Dutch	No	*M. avium* *S. paratyphi*	Q32X (homozygous)
2	Dutch	No	*M. avium* *Salmonella* sp.	Q376X (homozygous)
3	Turkish	Yes	*M. bovis* BCG *S. typhimurium*	del409–549 (homozygous)

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on cells from patients and controls (Fig. 2A).

The combination of CD2 mAb with exogenous recombinant IL-12 (rIL-12) synergized in inducing proliferation and IFN-γ production by PBMCs from healthy individuals (Fig. 2, B, C, and E). In contrast, rIL-12 failed to synergize with CD2 mAb in inducing proliferation or IFN-γ production in patients' PBMCs (Fig. 2, B, C, and E). rIL-2, however, synergized with CD2 mAb in inducing proliferation of patients' PBMCs (Fig. 1), showing that patients' PBMCs generally are not defective in their capacity to respond to cytokines. IL-12 also could not induce tyrosine phosphorylation of the IL-12R–associated signal transducer and activator of transcription STAT-4 in patients' cells (*24*).

Only small amounts of IFN-γ were produced by patients' cells when triggered by the mitogenic combination of $CD2^+$ and CD28 mAbs. However, this could not be inhibited by neutralizing IL-12 mAb; thus, the low release of IFN-γ in these patients is independent of endogenous IL-12 (Fig. 2, D and E) (*21*). In contrast, IFN-γ release in control cultures was reduced by >70% in the presence of saturating concentrations of mAbs to IL-12 (Fig. 2, D and E) (*21*). Although IL-12 is a major promoter of IFN-γ production (*1*, *2*), some IFN-γ can be produced in the absence of IL-12 or IL-12Rβ1 in IL-12 $p40^{-/-}$ (*25*) and IL-12Rβ$1^{-/-}$ mice (*26*). Although it is unclear how such a pathway is regulated (*27*, *28*), the results reported here likely reveal a similar pathway in humans. This IL-12R–independent pathway of (low) IFN-γ production probably accounts for the lack of enhanced IL-4 production in our patients (*29*).

Collectively, these results show that these three patients do not express functional IL-12R complexes and that their remaining T cell–dependent IFN-γ production is independent of endogenous IL-12.

cDNA and genomic IL-12Rβ1 DNA sequence analysis identified distinct genetic mutations. Patient 1 was homozygous for a nonsense mutation at nucleotide position 94 (C → T) at the cDNA and the genomic level. Both parents were heterozygous, which indicates an autosomal recessive segregation pattern. This mutation introduces a premature stop codon in the extracellular domain (Table 1). Patient 2 appeared homozygous for a nonsense mutation at nucleotide position 1126 (C → T) both at the cDNA and the genomic DNA level. This mutation also results in a premature stop codon in the extracellular domain. Again, both parents were heterozygous (*30*). In patient 3, a deletion was found in the cDNA that extended from nucleotide position 409 to position 549. This deletion led to a frameshift that introduced a premature stop codon at nucleotide positions 570 to 572 (TGA) in the extracellular domain of the IL-12Rβ1 gene. Both parents were heterozygous for this deletion, whereas the patient's healthy sibling

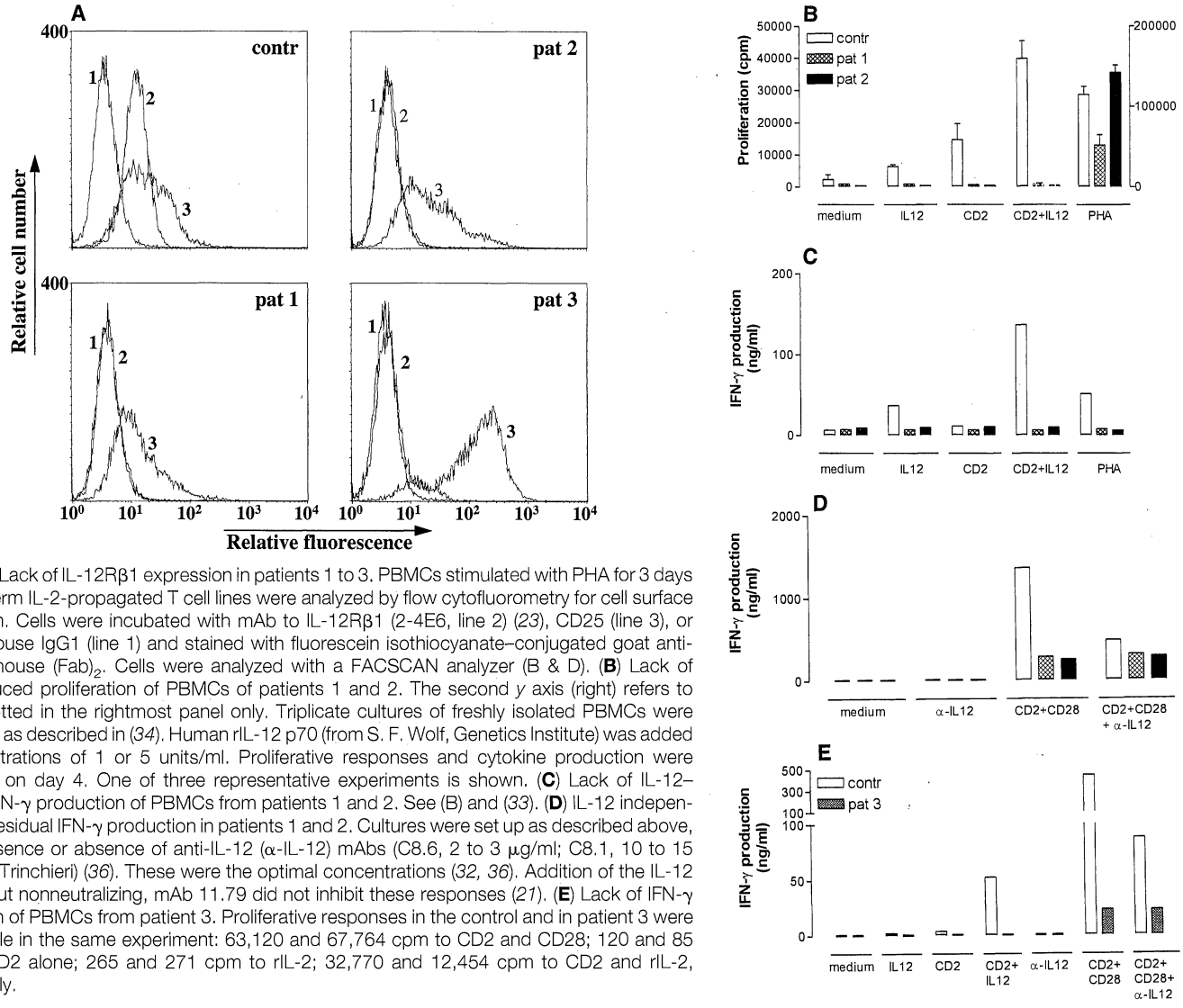

Fig. 2. (**A**) Lack of IL-12Rβ1 expression in patients 1 to 3. PBMCs stimulated with PHA for 3 days or short-term IL-2-propagated T cell lines were analyzed by flow cytofluorometry for cell surface expression. Cells were incubated with mAb to IL-12Rβ1 (2-4E6, line 2) (*23*), CD25 (line 3), or control mouse IgG1 (line 1) and stained with fluorescein isothiocyanate–conjugated goat antibody to mouse $(Fab)_2$. Cells were analyzed with a FACSCAN analyzer (B & D). (**B**) Lack of IL-12–induced proliferation of PBMCs of patients 1 and 2. The second *y* axis (right) refers to results plotted in the rightmost panel only. Triplicate cultures of freshly isolated PBMCs were stimulated as described in (*34*). Human rIL-12 p70 (from S. F. Wolf, Genetics Institute) was added at concentrations of 1 or 5 units/ml. Proliferative responses and cytokine production were measured on day 4. One of three representative experiments is shown. (**C**) Lack of IL-12–induced IFN-γ production of PBMCs from patients 1 and 2. See (B) and (*33*). (**D**) IL-12 independence of residual IFN-γ production in patients 1 and 2. Cultures were set up as described above, in the presence or absence of anti-IL-12 (α-IL-12) mAbs (C8.6, 2 to 3 μg/ml; C8.1, 10 to 15 μg/ml; G. Trinchieri) (*36*). These were the optimal concentrations (*32*, *36*). Addition of the IL-12 binding, but nonneutralizing, mAb 11.79 did not inhibit these responses (*21*). (**E**) Lack of IFN-γ production of PBMCs from patient 3. Proliferative responses in the control and in patient 3 were comparable in the same experiment: 63,120 and 67,764 cpm to CD2 and CD28; 120 and 85 cpm to CD2 alone; 265 and 271 cpm to rIL-2; 32,770 and 12,454 cpm to CD2 and rIL-2, respectively.

was unaffected, which conforms to an autosomal recessive segregation pattern (Table 1) (*30*). This deletion most probably results from a splice mutation that leads to the skipping of one exon, but one of the flanking introns could not be amplified.

The six parents as well as the tested healthy siblings of the three patients all expressed functionally competent IL-12R complexes (*21*).

The genetic lack of IL12-Rβ1 expression represents a human immunodeficiency and is sufficient to explain the observed phenotypes in all three patients. The defect in IFN-γ production and the extreme susceptibility to mycobacterial and *Salmonella* infections in these patients most probably result directly from the lack of IL-12R expression and signaling. The selective susceptibility to mycobacterial and *Salmonella* infections shows that the type-1 cytokine pathway is essential for controlling resistance to these pathogens and that no other redundant protective immune mechanism can compensate for this deficiency in these patients. The patients did not develop any abnormal infections with other viral, bacterial, or fungal pathogens (*18*), which suggests that IL-12 may be dispensable for protection to pathogens other than intracellular bacteria.

It is not yet known how many nonfunctional alleles have been maintained in the human population (*31*) and whether partially defective IL-12R alleles also exist, as described for the IFN-γR1 gene (*15*). Such allelic variants could contribute to genetically controlled disease susceptibility not only in infectious diseases but also in other conditions that rely on IL-12R signaling, such as cancer. The definition of unusual immunodeficiencies reveals fundamental insights into cytokine and cellular effector pathways of protective immunity and may allow the design of appropriate and effective therapeutic regimens.

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17. Histological analysis of lymph node material from patient 3 during the BCG infection episode revealed a tuberculoid-like organization with clear granulomatous structures. Ziehl Neelsen staining revealed the presence of many mycobacteria staining with acid fast.
18. Patients all tested negative for human immunodeficiency virus, had responded normally to routine early childhood vaccinations, and had no history of any abnormal viral, bacterial, or fungal infections other than the ones reported here. With regard to previous viral infections, patient 1 had immunoglobulin G (IgG) antibodies to cytomegalovirus (CMV), varicella zoster virus, and Epstein–Barr virus (EBV). Patient 3 had IgG antibodies to CMV and EBV. Patient 2 was seronegative for these viruses. All three patients were from the Netherlands. In patient 1, both infections could be treated effectively with an extensive course of antibiotic therapy. In patient 2, all episodes could be treated well with antibiotics, which were supplemented with rIFN-γ during the last year on the basis of findings reported in this study. Also in patient 3, both infections could be treated succesfully with antibiotics, and axillar lymph nodes containing large numbers of BCG were removed surgically. There was no anamnestic or genetic evidence for consanguinity in the families of patients 1 and 2. In contrast, the parents of patient 3 were of consanguineous descent. Patient 1 had one full sister, patient 2 had two full brothers, and patient 3 had one full brother. All patients' siblings and parents were unaffected. The patients were unrelated to those described in (*31*). Detailed case reports, including immunological and other analyses, will be described elsewhere.
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22. R. de Jong and T. H. M. Ottenhoff, unpublished data. TNF-α induction experiments were performed as described in (*10*) with minor modifications.
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24. PBMCs of patients and controls were stimulated with PHA and rIL-2 and incubated with rIL-12 (10 units/ml) or control culture medium for 45 min. Cells were lysed in 10 mM tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 0.25% sodium deoxycholate [containing aprotinin (10 μg/ml), leupeptin (10 μg/ml), 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 10 mM NaF] and immunoprecipitated with rabbit antisera to STAT-4 (Santa Cruz Biotechnology, CA), and precipitates were resolved by SDS–polyacrylamide gel electrophoresis. After transfer to nitrocellulose, blots were probed with labeled antibody to phosphotyrosine and phosphorylated tyrosine residues were visualized by enhanced chemiluminescence. IL-12–induced phosphorylation was detectable in IL-12–stimulated control T cells but not in patients 1 and 2 (patient 3 could not be tested). Equal loading of STAT-4 was shown by stripping the same membranes and reprobing them with polyclonal antiserum to STAT-4, followed by incubation with horseradish peroxidase–labeled horse immunoglobulin to rabbit (CLB, the Netherlands). Bands were visualized as described above.
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29. IL-4 was determined by a specific enzyme-linked immunosorbent assay (ELISA) with a lower limit of detection of 0.6 pg/ml (CLB Pelikine Compact, Amsterdam, the Netherlands). The same supernatants as in Figs. 1 and 2 were tested. Experiments were repeated at least twice.
30. Extraction of total RNA from PBMCs or EBV-transformed B cell genomic DNA, preparation of cDNA, and polymerase chain reaction (PCR) were performed as in (*15*). Primers for the amplification of the IL-12Rβ1 cDNA coding region were sense 5′-TGAACCTCGCAGGTGGCAGA-3′ and antisense 5′-TCGGGCGAGTCACTCACCCT-3′ (*1*). Sequencing was done with an Abi Prism dRhodamine terminator kit and analyzed with an Abi Prism model 377 DNA sequencer (Perkin-Elmer Applied Biosystems). A series of primers based on the published cDNA sequence was prepared for genomic amplification of the mutations in patients 1 and 2. Intrafamilial segregation of the mutation in kindred 1 was obtained by genomic sequencing. In kindred 2, the mutation alters an Eco RII restriction site and intrafamilial segregation was therefore analyzed by Eco RII digestion of genomic PCR products surrounding nucleotide position 1126. cDNA analysis was performed for patient 3, including intrafamilial cDNA segregation of the deletion. The analysis of a PCR fragment encompassing the deleted sequence (forward primer, CTGTGCTGTACACTGTCACA; reverse primer, TGGGTTGGCTGCTCTTTCAG) revealed a unique fragment of 475 base pairs (bp) (wild type) for the homozygous brother, a wild-type 475-bp and a mutant 335-bp heterozygous profile for both parents, and a homozygous mutant 335-bp (deletion) band for the patient. The deleted fragment is 140 bp long and extends from position 409 to 549.
31. F. Altare *et al.*, *Science* **280**, 1432 (1998).
32. R. de Jong *et al.*, *J. Immunol.* **159**, 786 (1997).
33. PBMCs were isolated from heparin-treated blood and plated at 1.5×10^5 cells per well in triplicate cultures in 96-well flat-bottomed plates (Costar) in Iscove's modified Dulbecco's medium (IMDM; Gibco) supplemented with 10% human pooled serum for proliferation assays or with 10% fetal calf serum (Gibco) for cytokine production analysis. PBMCs were stimulated with PHA (1 μg/ml) (Wellcome), a combination of CD2 mAb (CLB-T11.1/1 and CLB-T11.2/1) (5 μg/ml) in the absence or presence of CD28 mAb (CLB-CD28/1) (5 μg/ml) (all obtained from RAW van Lier, CLB, Amsterdam). rIL-2 (10 units/ml) (Eurocetus) was added as indicated. To measure proliferation, we pulsed cultures with 0.5 μCi of [^{3}H]thymidine (Du Pont de Nemours) and harvested them on day 4.
34. Day 4 supernatants were harvested from parallel cultures and measured for IFN-γ production by ELISA, using the 4SB3 mAb (European Collection of Animal Cell Cultures) for coating and biotinylated MD-1 mAb as a secondary mAb to IFN-γ [references in (*32*)]. Culture supernatants were assayed in serial dilutions and cytokine concentrations were determined by interpolation of values in the linear range of the standard curves. Lower limit of detection was 0.5 ng/ml.
35. Whole blood cultures were stimulated with LPS (Difco) (100 ng/ml) or *M. avium* (20 μg/ml) in the presence or absence of rIFN-γ (Boehringer) (500 units/ml), for 18 hours and supernatants were assayed for IL-12 p70. Whole blood was collected in sterile tubes containing sodium heparin (Endotube ET, Chromogenix). To prevent spontaneous production of cytokines by endotoxin(like) substances, IMDM was ultrafiltrated by a hollow fiber dialyzer (Hemoflow F40S). Triplicate cultures of whole blood containing 2.5×10^5 PBMCs per milliliter were set up in ultrafiltrated IMDM supplemented with sodium heparin (50 international units/ml) (Leo Pharmaceutical Products). We assayed supernatants for IL-12 p70 release by ELISA (R&D systems) (lower limit of detection, 0.5 pg/ml).
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38. We thank R. A. W. van Lier, D. Presky, and G. Trinchieri for providing mAbs; S. F. Wolf for providing human rIL-12, G. van Schijndel for advice in signaling experiments; E. A. Wierenga for reverse transcriptase–PCR and discussions; J. Schrander for arranging materials; H. T. Spierings for figures; E. Zanelli for discussion; R. R. P. de Vries and R. Van Furth for continuous support and discussions; and E. Zanelli, R. R. P. de Vries, F. Koning, and C. J. M. Melief for reviewing the manuscript. Supported by the Netherlands Leprosy Relief Association, the Amsterdam/Leiden Institute for Immunology, Macropa Foundation, the Commission of the European Communities, the Fondation Marcel Merieux, INSERM, AFM, and PHRC.

29 December 1997; accepted 31 March 1998