progress toward an answer may be achieved by comparing the surface composition of the KBO 1993 SC (*19*) with that of 1997 CU26. The spectrum of 1993 SC shows no evidence for water ice unlike that of 1997 CU26 but instead shows evidence for light hydrocarbons such as methane, ethane, ethylene, or acetylene (*19*). This suggests among other possibilities that some of the existing Centaurs have undergone surface modification since they left the Kuiper belt. It also supports the idea that the KBOs may be a compositionally diverse group of objects (*20*, *21*).

Considering only solar system dynamics, it is difficult to escape the conclusion that the Kuiper belt is the source of the Centaurs (*2*, *3*). Thus, the idea that at least some of the Centaurs have undergone substantial surface modification since leaving the Kuiper belt is attractive. One possible mechanism for such surface modification would be preferential sublimation of more volatile species, leaving less volatile species behind as a lag deposit. At the roughly 90 to 95 K present average surface temperature of an object like 1997 CU26 (*11*), one would expect that light hydrocarbons would be preferentially lost whereas water ice and heavier hydrocarbons would be retained. In addition, the closer proximity to the sun of the Centaurs relative to their presumed genesis zone would result in an increased flux of solar ultraviolet radiation on their surfaces with an accompanying increase in the rate of surface photochemical reactions. Those processes would tend to convert light hydrocarbons to heavy hydrocarbons (*13*), increasing any existing surface stock of heavy hydrocarbons. If the Kuiper belt is indeed the source of the short-period comets (*2*, *3*), then the possible presence of heavy hydrocarbons on the Centaurs, like the short-period comets (*20*), would at least be consistent with their originating in the Kuiper belt.

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11. The optical constants of water ice used here were taken from (*9*) and shifted in wavelength to agree with (*10*). The data of (*9*) were deemed to be more precise in the 1.4- to 2.4-μm wavelength region than those in (*10*), but the central wavelengths of the absorption bands in (*10*) corresponded more closely with those of water ice at the 90 to 95 K estimated average present surface temperature of 1997 CU26. This temperature range was calculated assuming a spherical body, a 5 to 20% bolometric bond albedo, instantaneous equilibrium with sunlight, a mean solar zenith angle of $\sqrt{2/\pi}$, and a heliocentric distance of 13.7 AU.
12. The optical constants for the red material were derived by assuming a real index of refraction of 1.4 at a wavelength of 1.0 μm and a Lambert absorption coefficient that increased linearly toward longer wavelengths. These quantities were processed through a subtractive Kramers-Kronig algorithm giving the real indices of refraction corresponding to the defined imaginary indices and the real index in the visual. The calculated reflectance for 10-μm grains gives a roughly linear increase in reflectance from 7.5% at 1.0 μm to 9% at 2.5 μm
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15. The convolution was done as a direct, discrete, numerical-integral convolution (not as a Fourier convolution). The purpose is to model data obtained at intrinsically lower spectral resolution (with the associated increase in SNR due to the larger spectral bandpass). In contrast to the common practice of binning data, which is mathematically equivalent to a discrete convolution with a square wave, the bandpass of most grating and grism spectrometers is very nearly Gaussian; thus, the result is a much better approximation to spectral data obtained at intrinsically lower spectral resolution.
16. The uncertainties were estimated by propagating the error bars of the high-resolution data directly through the convolution (*15*). Photon shot noise from the sky background is expected to be the dominant noise source, but, because the contributions of all the noise sources are not known, a correction for the photon shot noise difference expected in the wider bandpass of the convolved data was not included in the calculation.
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15 January 1998; accepted 8 April 1998

Impairment of Mycobacterial Immunity in Human Interleukin-12 Receptor Deficiency

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In humans, interferon γ (IFN-γ) receptor deficiency leads to a predisposition to mycobacterial infections and impairs the formation of mature granulomas. Interleukin-12 (IL-12) receptor deficiency was found in otherwise healthy individuals with mycobacterial infections. Mature granulomas were seen, surrounded by T cells and centered with epithelioid and multinucleated giant cells, yet reduced IFN-γ concentrations were found to be secreted by activated natural killer and T cells. Thus, IL-12–dependent IFN-γ secretion in humans seems essential in the control of mycobacterial infections, despite the formation of mature granulomas due to IL-12–independent IFN-γ secretion.

Bacille Calmette-Guérin (BCG) and nontuberculous mycobacteria (NTM) are poorly virulent mycobacteria that may cause disseminated disease in otherwise healthy children (*1*–*3*). The identification of inherited IFN-γ receptor ligand-binding chain (IFN-γR1) deficiency provided the first genetic etiology for this syndrome (*4*) and highlighted the importance of IFN-γ, a pleiotropic cytokine secreted by natural killer (NK) and T cells (*5*), in the control of mycobacteria in humans. The lack of mature mycobacterial granulomas showed that their formation is strictly IFN-γ–dependent. Partial, as opposed to complete, IFN-γR1 deficiency is associated with mature granulomas and a milder course of mycobacterial infection (*6*). However, a number

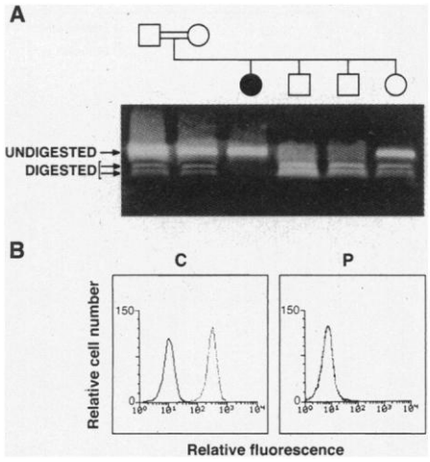

Fig. 1. A nonsense recessive mutation in the IL-12Rβ1 gene. (**A**) Segregation of the genomic mutation in kindred 1, as shown by 2% agarose gel electrophoresis of Mbo II digests of the amplified IL-12Rβ1 gene around position 913. The Mbo II site in the wild-type sequence is altered by the mutation (GAAGA → GTAGA); the overrepresentation of undigested products in heterozygous carriers is attributable to the presence of heteroduplex molecules after amplification. (**B**) Flow cytometry analysis of IL-12Rβ1 on PHA-activated *Herpesvirus saimiri*–transformed T cells with specific mouse mAb 12Rb.3F12 (dashed lines), compared with an isotypic control (black lines), in a control (C) and in patient 1 (P).

Table 1. Patients with inherited IL-12Rβ1 deficiency.

Patient	Origin	Consanguinity	Infection	Mutation*
1	Morocco	Yes	BCG, *S. enteritidis*	K305X†
2	Turkey	Yes	BCG	783+1G → C‡
3	Cyprus	Yes	*M. avium*, *S. enteritidis*	Q214R§
4	Cyprus	Yes	*M. avium*	Not identified

*Mutations are designated according to (*31*). †Nonsense mutation AAG → TAG at nucleotide position 913 results in the substitution of a lysine (K) by a stop codon (X) at amino acid position 305 and is designated as K305X; it causes the interruption of the coding region upstream of the transmembrane segment, precluding surface expression of the receptor, as detected on T cells by flow cytometry with specific antibodies. The mutation is homozygous in the patient only, and both parents are heterozygous. ‡Splice mutation G → C at the first intronic nucleotide after the exon spanning nucleotides 701 to 783 causes skipping of this exon, as detected by cDNA-PCR, and is designated as 783+1G → C; exon skipping causes a frame shift that leads to premature termination of translation before the transmembrane segment (TAA at nucleotides 879 to 881), precluding expression of the receptor, as detected on T cells by flow cytometry with specific antibodies. The mutation is homozygous in the patient only, and both parents are heterozygous. §Missense mutation CAG to CGG at nucleotide position 641 results in the substitution of a glutamine by an arginine at amino acid position 214 and is designated as Q214R; in patient 3, it is likely to be pathogenic, as attested by the lack of detectable IL-12Rβ1 on the surface of activated T cells by flow cytometry with two specific mAbs. No mutation could be sought in deceased patient 4, the brother of patient 3, yet he is likely to share the homozygous Q214R mutation; no material was available from the deceased father and the healthy sisters, but the mother is heterozygous for the mutation.

of disseminated BCG and NTM infections remain unexplained (*7*).

Four patients from three unrelated kindreds were investigated in this study (*8*). All suffered from disseminated mycobacterial infections attributable to BCG or *Mycobacterium avium*, and two of them also had non-typhi salmonella infections. No well-defined immunodeficiency could be detected in these otherwise healthy children, and the diagnosis of IFN-γR1 deficiency was excluded (9). Mutations in IFN-γ and in IFN-γR1–associated molecules within the receptor complex (*10*), as well as mutations in either of the two IL-12 p70 subunits (p35 and p40), a potent IFN-γ–inducing heterodimeric cytokine secreted by dendritic cells and phagocytes (*11*), were also excluded.

Mutations in each of the two IL-12 receptor subunits (β1 and β2), expressed on NK and T cells (*12*), were sought. A nonsense nucleotide substitution (AAG → TAG) at position 913 of the IL-12Rβ1 cDNA coding region was identified in patient 1 (*13*) (Table 1). The patient was found to be homozygous for the corresponding genomic mutation, which was inherited as an autosomal recessive trait in the kindred (*14*) (Fig. 1A). Two specific monoclonal antibodies (mAbs) failed to detect IL-12Rβ1 molecules at the cell surface of phytohemagglutinin (PHA)–activated peripheral blood T cells and PHA-activated *Herpesvirus saimiri*–transformed T cells (*15*) (Fig. 1B). Recessive mutations precluding expression of IL-12Rβ1 were also identified in the other two kindreds (Table 1).

There were normal numbers of NK cells in the blood of patient 1, and the efficient destruction of K562 cells by peripheral blood mononuclear cells (PBMCs) attested that NK cells were also functional (Fig. 2A) (*16*). Addition of recombinant IL-12 did not further up-regulate the cytotoxicity of the patient's NK cells, whereas it did up-regulate the cytotoxicity of control NK cells; this confirmed that IL-12Rβ1 deficiency results in impaired IL-12 receptor function (*12*). However, efficient NK cytotoxicity was accompanied by secreted IFN-γ concentration only 1% of that of control activated NK cells (Fig. 2B). Impairment of IFN-γ secretion by otherwise functional NK cells also occurs in mice genetically deprived of IL-12p40 or IL-12Rβ1; these mice are termed IL-12 knockout (IL-12KO) mice (*17*).

When T cells from the patient were incubated with PHA (*18*), they produced only 5% of the IFN-γ of control cells (Fig. 2B). Normal mitogen-driven T lymphocyte proliferation in vitro showed that impaired IFN-γ secretion did not result from impaired T cell activation (Fig. 2C). Secretion of IFN-γ from tuberculin-activated T lymphocytes was reduced by 99% relative to control T cells. This finding implies that most specific T cells primed in vivo by mycobacterial antigens, in the absence of costimulation by IL-12, could not secrete normal amounts of IFN-γ when restimulated. Impairment of IFN-γ secretion by otherwise functional T cells also occurs in IL-12KO mice (*17*).

Tuberculin-specific delayed-type hypersensitivity (DTH) (*19*) was normal in BCG-infected patients with IL-12Rβ1 deficiency, as seen in patients with IFN-γR1 deficiency (*4*). This further suggests that the cooperation between antigen-presenting cells and tuberculin-specific memory T cells in vivo was not globally affected by the lack of IL-12 stimulation, which in turn implies that neither IL-12 nor IFN-γ are essential for DTH in humans. Antigen-specific DTH in IL-12KO mice seems somewhat impaired, however (*17*).

Paucibacillary, well-circumscribed granulomas with epithelioid and giant multinucleated cells were identified in the lymph nodes and liver of BCG-infected patient 1 (Fig. 3), unlike in BCG-infected tissue from children with complete IFN-γR1 deficiency (*20*). Immunostaining revealed CD3-positive lymphocytes, including mostly helper ($CD4^+$ and $CD45RO^+$) and smaller numbers of cytotoxic ($GMP\text{-}17^+$, $CD8^+$, and $CD45RO^+$) memory T cells, as in control children with benign local BCG infection (BCG-itis) (*20*). Thus, even though the

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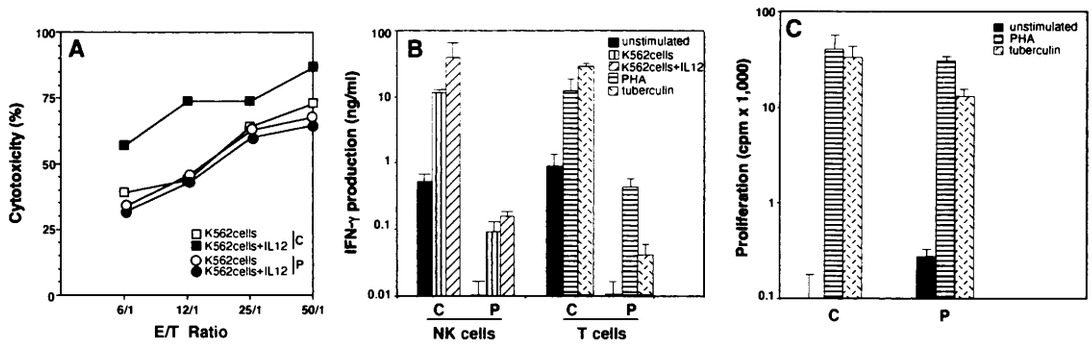

Fig. 2. Impaired IFN-γ secretion by NK and T lymphocytes. (**A**) NK cells from a control individual (C) and patient 1 (P) were tested for natural cytotoxicity against K562 cells alone or in the presence of recombinant IL-12. A single representative experiment is shown; the experiment was done twice. E/T, effector/target ratio. (**B**) Production of IFN-γ from NK cells, tested under the same conditions as in (A), and T cells stimulated by PHA or tuberculin from a control individual and patient 1; means of two experiments are shown. (**C**) T cells were also tested under the same conditions for proliferation.

kinetics of BCG granuloma formation or the ability to develop mature granulomas in response to other mycobacterial species were not determined, mature BCG granulomas were seen in the absence of IL-12–mediated immunity. Lung granuloma formation is impaired in IL-12KO mice infected with *Mycobacterium tuberculosis* (*21*).

IL-12KO mice are highly susceptible to *M. tuberculosis* (*21*), whereas infections with less virulent mycobacteria, such as BCG and NTM, have not been reported to date. In humans, IL-12 seems important in protective immunity to *M. tuberculosis* (*22*), *M. avium* (*23*), and *M. leprae* (*24*). Evidence from the four patients in the present study (who were genetically deprived of IL-12–mediated immunity) reveals that IL-12 is irreplaceable for protective immunity to even poorly pathogenic mycobacteria. It is likely that IL-12 is also essential in the control of more virulent mycobacterial species, and perhaps other, milder IL-12Rβ1 mutations may lead to a predisposition to clinical tuberculosis in the general population (*25*).

IL-12KO mice were also found to be highly susceptible to *Leishmania major* infection (*26*). Much experimental evidence suggests that IL-12–mediated immunity may be important in the control of a wide range of viral, bacterial, and parasitic microorganisms in mice and humans (*27*). However, despite probable exposure to most childhood pathogens and environmental microorganisms, patients with IL-12Rβ1 deficiency did not suffer from infections due to microbes other than mycobacteria (and, to a lesser extent, salmonella); this suggests that IL-12 in humans is not necessary to control most other infections. Three other patients sharing this phenotype are described in an accompanying report (*28*), but the identification of more kindreds is probably needed to better appreciate the range of potential pathogens.

The selective susceptibility to mycobacterial infections is shared by IL-12Rβ1–deficient and IFN-γR1–deficient children (*29*). Two cytokines, IFN-γ and IL-12, appear to play both a selective and an essential role in human defense against mycobacteria. However, the clinical phenotype shared by children with each of these two genotypes is likely to arise from a single pathogenic mechanism. First, in IL-12Rβ1–deficient patients, IFN-γ production by otherwise functional NK and T lymphocytes is markedly impaired. Second, therapeutic use of IFN-γ cured the mycobacterial infection in IL-12Rβ1–deficient patient 3 (*8*). Insufficient IFN-γ production thus appears to be the main pathogenic mechanism in IL-12Rβ1–deficient patients.

Mycobacterial infections in IL-12Rβ1–deficient patients tend to have a milder course than in children with complete IFNγR1 deficiency (*4*), and such patients more closely resemble children with partial IFN-γR1 deficiency (*6*). Children with IL-12Rβ1 deficiency and partial IFN-γR1 deficiency have mature BCG granulomas, unlike children with complete IFN-γR1 deficiency. The milder phenotype is probably attributable to IL-12–independent pathways of IFN-γ production (*30*); residual amounts of IFN-γ were produced by IL-12Rβ1–deficient activated lymphocytes. The process of mature granuloma formation in response to mycobacterial infection is strictly IFN-γ–dependent. Although IL-12–dependent IFN-γ induction is not necessary for mature granuloma formation, it is essential for protective mycobacterial immunity in humans.

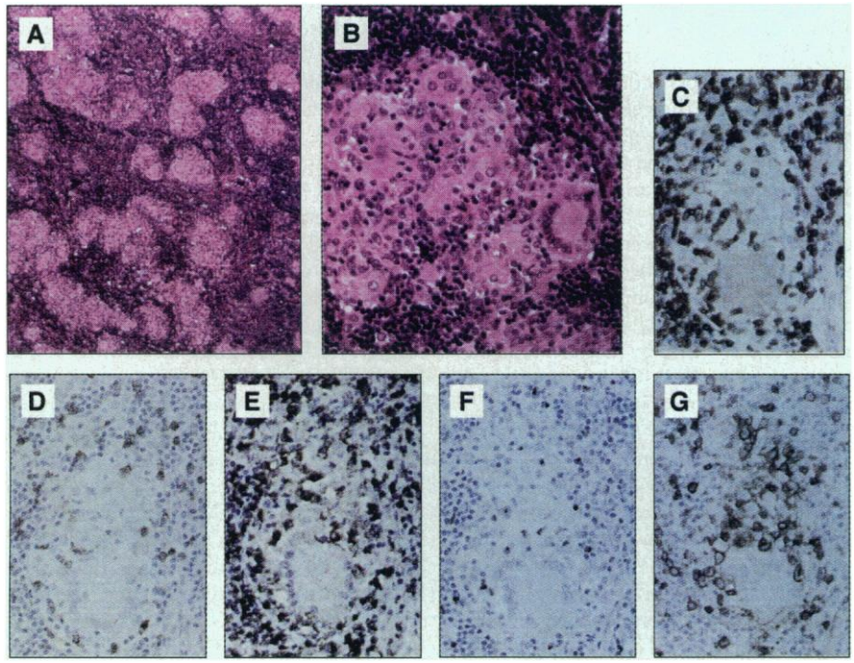

Fig. 3. Mature BCG granulomas. (**A** and **B**) Hematoxylin and eosin stainings of a BCG-infected lymph node from patient 1 at magnifications of 100× (A) and 400× (B). (**C** to **G**) Immunohistochemical stainings of 4-μm-thick serial sections of a granuloma from the same tissue sample with CD3- (C), CD8- (D), CD4- (E), GMP-17– (F), and CD45RO-specific (G) antibodies (magnifications, 400×).

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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′-TGAACCTCGCAGGTGGCAGA-3′ (sense) and 5′-TCGGGCGAGTCACTCACCCT-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