Letters to the Editor

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A Causative Relationship between Mutant *IFNgR1* Alleles and Impaired Cellular Response to IFNγ in a Compound Heterozygous Child

To the Editor:

Mutations in the *IFNgR1* gene have recently been identified in two consanguinous kindreds from Malta and Tunisia (Jouanguy et al. 1996; Newport et al. 1996). Affected children were found to be homozygous for two different alleles. No IFNγR1 protein molecules could be detected at the surface of peripheral blood cells, accounting for impaired IFNγ-mediated immunity. Patients had disseminated infections due to *Mycobacterium bovis*, bacille Calmette Guérin (BCG) vaccinal strain, or environmental nontuberculous mycobacteria (NTM), such as *M. avium, M. fortuitum,* and *M. chelonei* (MIM 209950). No other opportunistic infections were documented, with the possible exception of salmonellosis in one child, and four of five affected children died of mycobacterial infection. Thus, complete IFNγR1 deficiency appears to be an autosomal recessive immune disorder associated with severe and selective susceptibility to poorly pathogenic mycobacteria.

To better characterize this condition, it is important to demonstrate that there is a genuine causal relationship between mutant *IFNgR1* alleles and impaired cellular responses to IFNγ. It is also important to determine whether compound heterozygous mutations of the *IFNgR1* gene may be responsible for BCG or NTM infections in other kindreds (Casanova et al. 1995, 1996; Levin et al. 1995; Emile et al. 1997). We have therefore investigated an Italian child, born to nonconsanguinous parents, who presented at 3 years of age with disseminated infection due to *M. smegmatis* (Pierre-Audigier et al. 1997). The child was not vaccinated with BCG and died at 8 years of age of a progressive mycobacterial disease, despite intensive antimycobacterial therapy. Four older siblings had received the BCG vaccine in infancy with no adverse effect and were healthy.

To facilitate the genetic analysis of IFNγR1 deficiency, we first identified an intragenic polymorphic CA-repeat, designated "FA1" (fig. 1). The *IFNgR1* gene has been previously mapped to the broad chromosome region 6q16–6q22, by use of somatic cell hybrids (Pfizenmaier et al. 1988), and to 6q24.1–6q24.2, by in situ hybridization (Le Coniat et al. 1989; Pappanicolaou et al. 1997). The segregation of FA1 alleles within CEPH reference families provided a genetic mapping of FA1 between D6S292 (centromeric) and D6S1699 (telomeric) (4 cM). Physical mapping of the gene and surrounding markers on a YAC contig was then achieved. Intrafamilial genotyping showed that the affected child had a unique pattern for FA1 and the associated D6S1009, D6S310, and D6S1587 microsatellites, when compared with her four healthy siblings (not shown). This segregation was compatible with the diagnosis of autosomal recessive IFNγR1 deficiency.

Amplification of the seven *IFNgR1* exons and associated intronic consensus splice sites was performed for each member of the family. Sequencing revealed a 4-bp insertion, designated "107ins4," within *IFNgR1* exon 2 at one locus in the child and in the father (not shown). The four inserted nucleotides (TTAC) were found to duplicate flanking nucleotides 104–107, as has occasionally been observed with insertions causing other genetic diseases (Cooper and Krawczak 1991). The frameshift is expected to cause premature termination of translation before the transmembrane segment, because of a stop codon at nucleotides 115–117. A substitution of the first base of the consensus splice-donor site of *IFNgR1* intron 3 was found at the other locus in the child and in the mother, designated "200+1G→A" (not shown). This type of 5′ splice-site mutation is frequent in other genetic diseases and is expected to cause exon 2 skipping and/or cryptic splice-site usage (Krawczak et al. 1992). The affected child was the only member of the family carrying the two mutant alleles, further suggesting that he had IFNγR1 deficiency.

Analysis of *IFNgR1* mRNA in the patient's fibroblasts, by northern blot and amplification of the full-length *IFNgR1* cDNA coding region, failed to detect any alternative splicing product, when compared with healthy individuals (not shown). Likewise, amplification of the patient's cDNA, with primers specific for exon 1 (sense) and exon 3 (antisense), failed to detect exon 2 skipping and cryptic site usage. Even though the patient's *IFNgR1* mRNA appeared to be of normal molecular

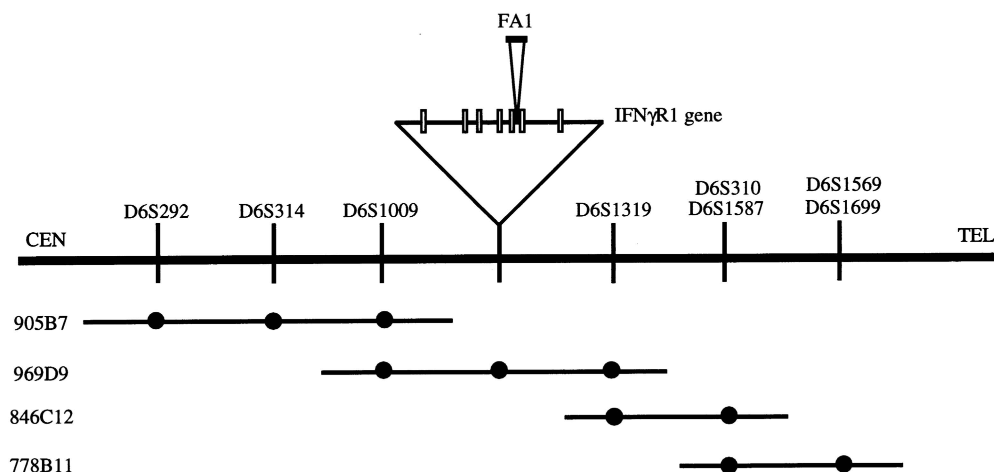

Figure 1 Genetic and physical map of the *IFNgR1* gene. Restriction digests of cosmids COSTCFP-2.8 and COSTCFP-7.13, carrying the *IFNgR1* gene (Merlin et al. 1997), were hybridized with a peroxydase-labeled (CA)30 probe. A specific fragment was found to contain a repeat consisting of 22 CAs located in the sixth intron (FA1; Genbank accession number U84721). When tested on 80 unrelated individuals, this marker was found to be highly informative, with 12 alleles and >90% heterozygosity. Linkage analyses were performed on three-generation CEPH families genotyped with polymorphic markers (Dausset et al. 1990). The FA1 marker was positioned on a framework consisting of the map of 5,264 markers (Dib et al. 1996), by use of the GMS algorithm (Lathrop and Lalouel 1988). On the basis of the best-supported order for the framework map, recombination fractions between adjacent markers were estimated using the LINKAGE program (Lathrop et al. 1984). The map was reevaluated until no further double recombination event could be eliminated. The FA1 microsatellite was thus genetically mapped between microsatellites D6S292 and D6S1699 (<4 cM). The CEPH YAC WC6.15 contig was previously reported with a series of polymorphic and nonpolymorphic markers (Dausset et al. 1992). Selected YACs of this contig were confirmed by PCR, to define a smaller contig encompassing the microsatellite FA1 and its flanking markers defined by the genetic mapping. The physical map of the region was then enriched by testing an additional panel of dinucleotide (Dib et al. 1996) and tetranucleotide (Sheffield et al. 1995) repeats on the same subset of YACs. The YAC contig consisting of 905B7, 969D9, 846C12, and 778B11 spans <4 Mb. All markers positioned on this contig are polymorphic CA repeats, except D6S1009 and D6S1003 (polymorphic tetranucleotide repeats) and D6S1319 (nonpolymorphic marker). The relative positions of D6S310 and D6S1587 and of D6S1569 and D6S1699 are not known.

weight and to be expressed at a normal level by northern blot, the patient's *IFNgR1* cDNA-PCR, however, was found to be 4 bp longer than the wild type. Moreover, direct sequencing of the amplicon confirmed that the only detectable *IFNgR1* mRNA species in this patient was encoded by the 107ins4 mutant allele. Together, these results suggest that the 107ins4 allele transcript is expressed at a normal level and that none of the 200+1G→A allele potential transcripts, including full-length, exon 2 skipping, and cryptic splice-site transcripts, is detectable.

We then analyzed cell surface expression of the receptor by flow cytometry with two IFNγR1-specific antibodies as reported elsewhere (Jouanguy et al. 1996). Whereas IFNγR1 molecules were present on the surface of peripheral blood mononuclear cells from healthy individuals, there were no detectable IFNγR1 molecules on the patient's cells (not shown). These data are consistent with the analysis of *IFNgR1* mRNA expression and strongly suggest that both the 107ins4 and 200+1G→A mutations preclude transcription of full-length wild-type mRNA and expression of detectable IFNγR1 molecules at the cell surface.

To document a functional defect in this patient, we took advantage of a previously reported method for the investigation of human leukocyte antigen (HLA)–class II deficiency (Lisowska-Grospierre et al. 1994) and analyzed the IFNγ-mediated induction of HLA-DR in fibroblasts. Concentrations of IFNγ, witin the range of 10–100,000 UI/ml, were tested for the induction of HLA-DR after 48 h and 72 h of incubation. In all conditions, there was no induction of HLA-DR on the patient's cells, as detected by flow cytometry, in contrast to the dramatic induction obtained with control cells after only 48 h in response to ⩾10 UI of IFNγ/ml (fig. 2*A*). Intracellular HLA-DR could also be detected by microscopic immunofluorescence in control cells but not in the patient's cells (not shown). These results suggested that the patient's fibroblasts were not responsive to IFNγ because of a lack of functional surface IFNγR1 molecules.

Finally, we attempted to demonstrate a causal relationship between the two mutant *IFNgR1* alleles identified and impaired cellular response to IFNγ. We thus transiently transfected the patient's fibroblasts with wild-type *IFNgR1* allele and assessed the induction of

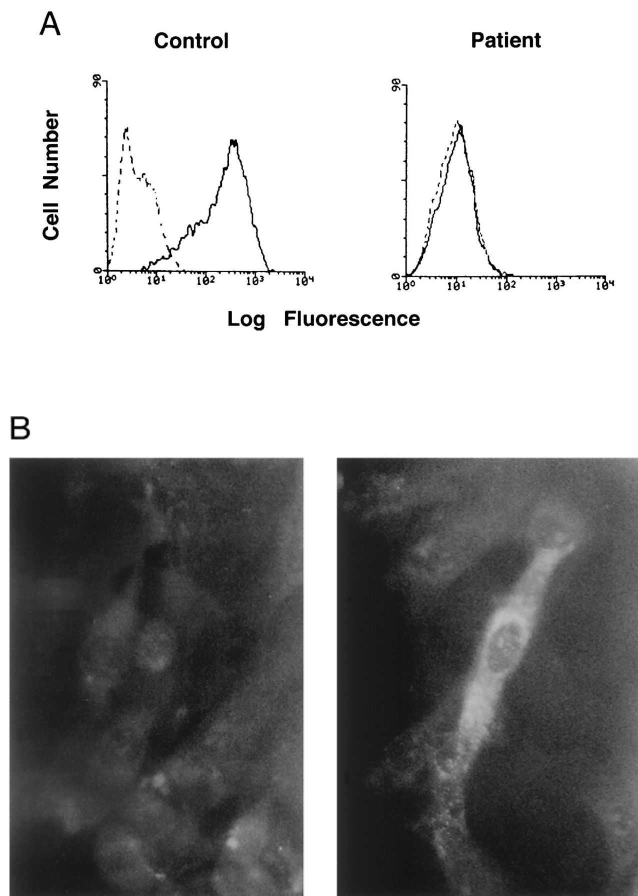

Figure 2 Impaired IFNγ-mediated induction of HLA-DR on the patient's fibroblasts and complementation with wild-type *IFNgR1* gene. *A,* Flow cytometry analysis of HLA-II on fibroblasts before (*dashed lines*) and after (*solid lines*) addition of IFNγ in a control (*left*) and the patient (*right*). An adherent fibroblastic cell line was established from a skin biopsy of the patient, grown in RPMI 1640 (Gibco BRL) supplemented with 10% FCS (Gibco BRL) (complete medium) and transformed with SV40T as described elsewhere (Lisowska-Grospierre et al. 1994). One million fibroblastic cells were treated with 200 IU recombinant human IFNγ/ml (Genex, Biogenex Laboratories) for 48 h, and the induction of HLA-DR was documented following trypsinization by flow cytometry with a fluorescein isothiocyanate–conjugated monoclonal antibody anti–HLA-DR (Becton Dickinson). *B,* Microscopic fluorescence analysis of IFNγ-induced HLA-II expression in the patient's fibroblasts transiently transfected with control plasmid (*left*) and with IFNγR1 expression vector (*right*). The 4×10^5 fibroblasts were transferred to sterile microscopic slides for in situ electrophoration in 100 μl of 10 mM sodium phosphate, pH 7.2, 1 mM $MgCl_2$, and 250 mM sucrose with 4 μg plasmid DNA (either an IFNγR1 expression vector referred to as "pSFFVhgR" or the vector with no insert, referred to as "pSFFVneo") (Farrar et al. 1991). Six pulses were delivered (5 ms, 320 V, 1 Hz, $E =$ 0.8 kV/cm) with a JOUAN GHT 128/A electropulser, and 5 min later the slides were placed in complete medium. After 3 h of culture, recombinant IFNγ was added at 10 IU/ml, and after 24 h the medium with IFNγ at the same concentration was replaced. Antibody binding (Bu27) on ethanol-fixed fibroblasts at 48 h following transfection was revealed ,as described elsewhere (Lisowska-Grospierre et al. 1994). Immunofluorescence analysis was carried out with a Leitz Orthoplan optical microscope.

intracellular HLA-DR in response to IFNγ, by microscopic immunofluorescence. In response to even low IFNγ concentrations (10 UI/ml) and after only a brief incubation (48 h), a number of cells intensely fluoresced, attesting that transfection of the *IFNgR1* gene conferred normal IFNγ-dependent induction of HLA-DR in the cytoplasm (fig. 2*B*). By contrast, transfection of the control vector was ineffective in inducing intracellular HLA-DR. Similar results were obtained with high concentrations of IFNγ (100,000 UI/ml) and 72-h incubation (not shown). Analysis of HLA-DR surface expression by flow cytometry was also consistent with these results (not shown).

We have thus demonstrated a cause-and-effect relationship between mutant *IFNgR1* alleles and impaired cellular response to IFNγ in this kindred. These data corroborate the results previously obtained for two kindreds and strengthen the association between inherited IFNγR1 deficiency and susceptibility to mycobacteria in affected children. This kindred provides further important information concerning IFNγR1 deficiency. The affected child was found to be the first compound heterozygous patient. Worthy of note, the two mutations found in this child were different mutations from the two identified in previous kindreds. The four mutations identified, to date, include a nonsense mutation, a deletion, an insertion, and a splice mutation. Thus, these data not only show that the disease is not limited to consanguinous families but also suggest that there is probably a relatively high allelic heterogeneity underlying inherited IFNγR1 deficiency.

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