Immunodeficiency and disseminated mycobacterial infection associated with homozygous nonsense mutation of IKKβ

To the Editor:

Nuclear factor kappa B (NF-κB) signaling is known to be important for host protection against infection. For activation, proteins of the NF-κB transcription factor must be released from constitutive interaction with inhibitory IκB proteins (IκBα, IκBβ, and IκBε), which sequester NF-κB complexes in the cytoplasm. This occurs through phosphorylation and degradation of IκB proteins by the upstream IκB kinase (IKK) complex, for example, following stimulation of cell surface receptors. The IKK complex consists of 2 catalytically active kinases, IκKα and IκKβ, and the regulatory subunit IκKγ, also known as NF-κB essential modulator (NEMO). In the canonical pathway of NF-κB activation, IκBα undergoes IκKβ-dependent phosphorylation and ubiquitin-mediated degradation, liberating the NF-κB heterodimer, which then translocates to the nucleus.1 Mutations in 2 proteins of the NF-κB signaling pathway, NEMO and IκBα, have been described in humans and result in immunodeficiency (ID), usually associated with ectodermal dysplasia (EDA). Hypomorphic hemizygote NEMO mutations cause X-linked EDA-ID, while hypermorphic IκBα mutations lead to an autosomal-dominant EDA-ID.2-4 Affected individuals are susceptible to severe infections with pyogenic bacteria and mycobacteria and in some cases opportunistic and viral pathogens.5 In addition, an autosomal-recessive mutation in IκKα has been associated with an in utero lethal Cocoon syndrome characterized by multiple fetal malformations.5

Here, we report an 18-month-old female, second child of first-degree consanguineous parents from the Arabian Peninsula (see Fig E1, A, in this article’s Online Repository at www.jacionline.org), who presented at age 2 months with oral ulcers and delayed separation of the umbilical cord, necessitating surgical removal. At age 3 months, she developed Salmonella sepsis and subsequently suffered severe recurrent infections caused by a range of organisms including Acinetobacter, Enterobacter, Stenotrophomonas, and Achromobacter species, rotavirus, and Candida. Chronic diarrhea and a generalized maculopapular rash were persistent from the neonatal period. Disseminated BCGosis was diagnosed from skin and gut biopsies, and antimycobacterial treatment started at age 4 months. Her family history was significant for a brother who had died at age 1 month from E coli sepsis and meningitis. In addition, 3 paternal grand uncles had died in infancy with short febrile illnesses.

At the age of 18 months, our patient had conical teeth, hepatosplenomegaly, and a severe skin rash (Fig 1, A and B). This was confirmed to be persistent BCGosis on skin biopsy showing a mixed inflammatory infiltrate and possible epithelioid granuloma formation (Fig E1, B), with acid-fast bacilli visible and a PCR positive for DNA of Mycobacterium tuberculosis complex, but negative for the esat-6 gene. Initial immunology investigations demonstrated normal proportions and numbers of naive and memory T, B, and natural killer cells, normal T-cell repertoire, and T-cell proliferation to PHA (Table I). Expression of MHC class II molecules was preserved. Neutrophil number, respiratory burst, and integrin expression were normal, as was phenotyping for monocytes and dendritic cell populations. Immunoglobulin levels had been documented to be low on repeated sampling at age 3 months (IgG 1.2 g/L, IgA < 0.05 g/L, and IgM < 0.05 g/L) and therefore immunoglobulin replacement had already been commenced. Liver function test results were normal (including aspartate transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase, albumin, total protein, and total bilirubin levels). Whole blood cytokine assays on 2 occasions demonstrated severely impaired production of IFN-γ to all stimuli, absent production of IL-17, and a markedly reduced production of the proinflammatory cytokines, TNF-α and IL-6, in response to a range of Toll-like receptor ligands (Fig 1, C). Stimulated IL-12 production was also reduced and not significantly rescued by addition of exogenous IFN-γ. The pattern of cytokine response and clinical features were not consistent with a classical IL-12/INF-γ pathway defect but would be consistent with a defect in the NF-κB pathway.

Our patient was treated with ethambutol, rifampicin, isoniazid, and pyridoxine as well as prophylactic azithromycin, fluconazole, and intravenous immunoglobulin in view of her immunodeficiency. INF-γ treatment was added at 50 μg/m² and gradually increased to 200 μg/m².2 This was not well tolerated, and she developed ulcerating skin inflammation and increasing hepatosplenomegaly necessitating low-dose steroid treatment (0.5 mg/kg) and therefore IFN-γ treatment was withdrawn. She continued to have recurrent episodes of fever and increased inflammatory markers that most likely reflected her BCGosis. She subsequently developed a progressive respiratory distress, was admitted to intensive care for noninvasive ventilation, and, unexpectedly, died from a massive gastrointestinal and pulmonary hemorrhage after a surgical central line insertion at age 25 months.

To identify the causative mutation, we used exome sequencing. Blood sample of the patient was obtained with informed consent from the parents in accordance with the Declaration of Helsinki and with approval from the ethics committees (04/Q0501/119 and 06/Q0508/16). Library preparation, exome capture, and sequencing have been done according to the manufactures instructions. For exome target enrichment, Agilent SureSelect 50 Mb kit was used. Sequencing was done using Illumina HiSeq with 94 bp paired-end reads. In the exome data, we found 22,754 single-nucleotide variants and small insertions/deletions, including 172 very rare ones, that is, those not seen in the 1000 Genomes database (April 2012 data release) and our internal databases. Five of these variants were homozygous (see Table E1 in this article’s Online Repository at www.jacionline.org). Among these we identified a homozygous nonsense mutation c.321C>A in the IKBKG gene that encodes the IKKβ protein, leading to a premature stop codon p.Y107X (Fig 1, D; see Fig E2, A, in this article’s Online Repository at www.jacionline.org). In the exome sequence data, we found no mutations in genes encoding IκBα, NEMO, or other proteins of the NF-κB pathway. Because gene IKBKG that encodes NEMO was poorly covered in the exome data, we studied it by Sanger sequencing and again found no mutations. We then confirmed the IKBKG mutation by
Sanger sequencing and showed that both parents were heterozygous carriers of this mutation (Fig E2, B). We then purified proteins from PBMCs and demonstrated that the patient completely lacked IKKβ expression (Fig 1, E). We used polyclonal rabbit anti-IKKβ antibody (Cell Signaling Technology cat no. 2678) produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu570 of the 756 amino acid IKKβ protein. Therefore, presence of a shorter IKKβ protein that potentially could be generated after reinitiation of translation seems unlikely. Further functional experiments were not possible, because no patient-derived cell lines were available after the patient’s death. Given that functional reconstitution experiments were impossible, we cannot formally prove that the IKKβ deficiency has caused the immune phenotype of the patient, although it is very likely in the absence of other candidate loss-of-function mutations. However, additional unknown modifier genetic or nongenetic factors may have affected the severity of the patient’s phenotype.

Here we demonstrate that an autosomal-recessive human IKKβ deficiency has a clinical presentation, which resembles both

**FIG 1.** A and B, Photographs showing conical teeth, hepatosplenomegally, and widespread rash. C, Quantitation of cytokine release from patient and healthy control PBMCs following in vitro activation with various stimuli (y-axis). Representative data from 1 of 2 repeat experiments are shown. D, Sequence data for the index case showing the homozygous c.321C>A mutation. E, Western blot showing that the IKKβ protein is present in the healthy donor (HD) but is absent from the patient’s (P) PBMCs.
TABLE I. Immunology investigations

| Cell type                  | Patient’s (age 20 mo) results | Age-matched control range |
|----------------------------|-------------------------------|---------------------------|
| White cell count           | $8.42 \times 10^3/L$          | $(5.0-15.0) \times 10^3/L$|
| Neutrophil count           | $3.87 \times 10^3/L$          | $(1.0-8.5) \times 10^3/L$  |
| Lymphocyte count           | $4.03 \times 10^3/L$          | $(3.0-13.5) \times 10^3/L$  |
| CD3 T cells                | $89.0\%$, $3.37 \times 10^3/L$| $39\%-73\%$, $(1.8-8.0) \times 10^3/L$ |
| CD19 B cells               | $7.0\%$, $0.27 \times 10^3/L$ | $17\%-41\%$, $(0.6-3.1) \times 10^3/L$ |
| CD16^{+} CD56^{+} natural killer cells | $3.0\%$, $0.11 \times 10^3/L$ | $3\%-16\%$, $(0.1-1.4) \times 10^3/L$ |
| CD3^{+} CD4^{+} T cells    | $61.0\%$, $2.31 \times 10^3/L$ | $25\%-50\%$, $(0.9-5.5) \times 10^3/L$ |
| CD3^{+} CD8^{+} T cells    | $27.0\%$, $1.02 \times 10^3/L$ | $11\%-32\%$, $(0.4-2.3) \times 10^3/L$ |
| Naïve T cells (CD4^{+}CD45RA^{+}CD27^{+}) | 90.0\% | 62%-90% |
| Naïve T cells (CD8^{+}CD45RA^{+}CD27^{+}) | 98.0\% | 46%-85% |
| Naïve B cells (CD19^{+}CD27^{+}) | 98.0\%* | 42.6%-82.3% |
| Nonsensewitched memory B cells (CD19^{+}IgD^{+}CD27^{+}) | 1.0\%* | 7.4%-32.5% |
| Class switched memory B cells (CD19^{+}IgD^{+}CD27^{+}) | 0.2\%* | 6.5%-29.1% |
| Transitional B cells (CD19^{+}IgM^{+}CD38^{−}) | 6.0\%* | 0.6%-3.4% |
| Plasmablasts (CD19^{+}CD38^{+}IgMwk) | 0.0\%* | 0.4%-3.6% |
| CD21 low B cells (CD19^{+}CD21wkCD38wk) | 2.0\% | 0.9%-7.6% |
| IgM/IgD B cells | Present | Present |
| IgG | 6.37 g/L after IVIG | 3.1-13.8 g/L |
| IgA | $<0.23$ g/L* | 0.3-1.2 g/L |
| IgM | 3.57 g/L* | 0.5-2.2 g/L |

IVIG, Intravenous immunoglobulin therapy.
*Abnormal result.

The severe granulomatous reaction seen in our patient may be a feature of systemic infection. However, altered keratinocyte function may additionally cause skin inflammation because mice with epidermis-specific deletion of IKKβ develop a severe TNF-mediated inflammatory skin disease. Our patient had chronic diarrhea, similar to most of the patients with the IkBα mutations. This is consistent with the importance of the NF-κB pathway for mucosal immunity, maintaining intestinal epithelia integrity and homeostasis in the gut. In line with other defects of NF-κB signaling, hypogammaglobulinemia and ectodermal dysplasia, characterized by peg teeth, were features of IKKβ deficiency in our case. The immunological phenotype was characterized by a profound defect in the production of proinflammatory cytokines despite intact development of all immune cell lineages. Although the clinical spectrum of the condition will be clarified by the identification of other affected families, the severity of immunodeficiency in our patient and failure to respond to conservative therapy suggest that bone marrow transplantation should be considered for children with complete IKKβ deficiency.
A case of partial dedicator of cytokinesis 8 deficiency with altered effector phenotype and impaired CD8+ and natural killer cell cytotoxicity

To the Editor:

Hyper-IgE syndrome (HIES) is a primary immunodeficiency characterized by recurrent infections predominantly involving the skin and lungs, chronic eczema, high serum IgE levels, and eosinophilia. HIES has diverse modes of inheritance. Autosomal-dominant HIES (Mendelian Inheritance in Man [MIM] no. 147060) is caused by mutations in the signal transducer and activator of transcription 3 gene encoding the dedicator of cytokinesis 8 (DOCK8) (MIM no. 243700), DOCK8 deficiency is characterized clinically by recurrent viral, fungal, and sinopulmonary infections.1-3 These patients also have severe atopic dermatitis, allergies, and an increased likelihood of developing cancer. DOCK8 deficiency affects both innate and adaptive immunity and causes combined immunodeficiency.

We present a patient who fulfilled clinical and immunological parameters for HIES (see the Case report section in this article’s Online Repository at www.jacionline.org). Mutations in the signal transducer and activator of transcription 3 gene were ruled out. At this stage, a DOCK8 deficiency was suspected. Using comparative genome hybridization, a heterozygous deletion of exons 2 to 8 was identified in the DOCK8 gene (see Fig E1, A, in this article’s Online Repository at www.jacionline.org.). Sequence analysis also demonstrated a heterozygous G > A mutation at position +5 in the 5′ splice donor site of intron 17 (IVS17+5 G>A) deleting the exon 17 (Genbank KC736820). This alteration corresponded to a deletion in the conserved DHR1 domain of the protein (Fig E1, B and C) and was absent in 50 healthy donors. Some normal DOCK8 transcripts were present, indicating that the exon 17 splicing mutation did not abrogate normal processing of this allele (Fig E1, D). DOCK8 transcript expression was lower in the patient than in controls in PBMCs, as well as CD4+ and natural killer (NK) cells (Fig E1, E); however, the DOCK8 protein was clearly detected in the patient by Western blot, albeit at reduced levels. On the basis of these findings, we conclude that this is a new case of partial DOCK8 deficiency (Fig E1, F).

Abnormalities in the T-cell compartment have been described in classical DOCK8 deficiency,4-6 and we decided to assess the impact of a partial DOCK8 deficiency on T-cell phenotype and function. Our patient showed defective thymopoiesis with an absence of T-cell receptor rearrangement excision circles, reduced recent thymic emigrants, a restricted T-cell repertoire, absence of T-cell receptor rearrangement excision circles, and decreased naive CD4 T cells in PBMCs (Table I; see Fig E1, B), thereby confirming the presence of an impact on thymic output.

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FIG E1. A, Genealogical tree for the kindred. Arrow highlights index case. Asterisks denote family members who died within the first 2 months of life with febrile illnesses. B, Histology showing granuloma in skin by hematoxylin and eosin staining.
FIG E2. A, Schematic IKKβ gene showing the mutation identified in our family. B, Sequence data for the index case, parents, and healthy control showing heterozygous carriage of the c.321C>A mutation in both parents.
| Chr | Position   | Ref/Obs | Transcript         | cDNA (change)            | Gene                                      | Amino acid |
|-----|------------|---------|--------------------|--------------------------|-------------------------------------------|------------|
| 1   | 109,276,144| —/T     | ENST00000370017    | c.2130_2131insT (frameshift insertion) | FNDC7 (fibronectin type III domain containing 7) | p.K710fs   |
| 1   | 116,280,898| C/T     | ENST00000261448    | c.G479A (nsSNV)          | CASQ2 (calsequestrin 2)                    | p.R160H    |
| 1   | 120,168,566| G/A     | ENST00000421812    | c.C158T (nsSNV)          | ZNF697 (zinc finger protein 697)           | p.P53L     |
| 2   | 216,197,149| A/G     | ENST00000236959    | c.A733G (nsSNV)          | ATIC (5-aminomidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase) | p.N245D    |
| 8   | 42,150,963 | C/A     | ENST00000520810    | c.C321A (stopgain SNV)   | IKBKB (inhibitor of kappa light polypeptide gene enhancer in B cells, kinase beta)         | p.Y107X    |