

Nuclear factor κ B essential modulator–deficient child with immunodeficiency yet without anhidrotic ectodermal dysplasia

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Background: Amorphic mutations in the X-linked nuclear factor κ B essential modulator (*NEMO*) gene cause Incontinentia pigmenti, which is lethal in hemizygous male patients. Hypomorphic *NEMO* mutations in male patients lead to anhidrotic ectodermal dysplasia (EDA) with immunodeficiency.

Objective: To report the clinical features of a child bearing a *NEMO* mutation who displayed an immunodeficiency without EDA.

Methods: Documentation of clinical care, chart review, standard immunologic and microbiological laboratory techniques, mutation analysis of the *NEMO* gene.

Results: Since the age of 15 months, the patient had *Mycobacterium avium* disease, beginning with multiple adenitis, later followed by disseminated osteomyelitis and dermatitis. In addition, *Haemophilus influenzae* and *Streptococcus pneumoniae* infections led to bronchiectasis. An immunologic work-up revealed a low production of IFN- γ by PBMCs associated with a hyper-IgM phenotype. Despite treatment using repeated cycles of a 4-drug antimycobacterial regimen, continuous subcutaneous IFN- γ , repeated antibiotic treatment, and intravenous immunoglobulin substitution, the boy remained chronically ill. At the age of 12 years, the disease was complicated by severe autoimmune hemolytic anemia and eventually fatal herpes simplex virus 1 encephalitis despite high-dose acyclovir therapy. Although he did not present any sign of EDA, a novel type of disease-causing hypomorphic *NEMO* mutation (110-111insC in exon 2) was identified.

Conclusion: This case demonstrates that patients hemizygous for *NEMO* mutations can present with an immunodeficiency

without EDA. An investigation of *NEMO* should thus be undertaken in selected children with immunodeficiency despite the lack of EDA. (J Allergy Clin Immunol 2004;114:1456-62.)

Key words: Children, hyper-IgM, immunodeficiency, ectodermal dysplasia, mycobacteria

Mutations in the gene encoding nuclear factor κ B (NF κ B) essential modulator (*NEMO*/IKK- γ) have recently been associated with X-linked disorders. The clinical phenotypes of the *NEMO* mutations differ according to the type, location, and extent of mutation.¹⁻³ All phenotypes described so far include ectodermal changes resulting in skin manifestations, except 1 case that is not described in detail.² Loss of function mutations in the *NEMO* gene cause X-linked dominant incontinentia pigmenti, which affects female patients.^{4,5} In male patients, mutations with large deletions in the *NEMO* gene are lethal in utero.

Hypomorphic mutations that impair but do not abolish *NEMO* function are found in male patients with X-linked ectodermal dysplasia and immunodeficiency (EDA-ID) or EDA-ID, osteopetrosis, lymphedema, and hemangiomas (OL-EDA-ID).⁶⁻¹¹ EDA-ID manifests as absent or conical shaped teeth, sparse scalp hair, and absence or rarity of sweat glands. There is an immunodeficiency resulting in infections of multiple sites including the digestive tract, respiratory tract, and skin, with the most common infectious agents gram-positive and gram-negative pyogenic bacteria and mycobacteria, but also *Pneumocystis jirovecii* and a few viruses. Some patients present with hyper-IgM syndrome (HIGM), and all show a very poor antibody response to polysaccharide antigens.

Here we report a *NEMO* mutation resulting in a novel phenotype presenting with an immunodeficiency but clearly without any signs of incontinentia pigmenti, anhidrotic ectodermal dysplasia, lymphedema, or osteopetrosis.

METHODS

Clinical data were gained from the clinical care of the patient as well as chart review. The clinical description is given in the text (see Results) and in Table I. The data of the immunologic work-up were obtained by standard immunologic and microbiological techniques unless indicated otherwise (Table II-IV). The detailed genetic

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Abbreviations used

DTH:	Delayed-type hypersensitivity
EDA:	Ectodermal dysplasia
EDA-ID:	Ectodermal dysplasia with immunodeficiency
EDAR:	Receptor for ectodysplasin
HIGM:	Hyper-IgM syndrome
HSV:	Herpes simplex virus 1
MRI:	Magnetic resonance imaging
NEMO:	Nuclear factor κ B essential modulator
NF κ B:	Nuclear factor κ B
OL-EDA-ID:	Ectodermal dysplasia with immunodeficiency, osteopetrosis, lymphedema, and hemangiomas
PPD:	Purified protein D
RANK:	Receptor activator of NF κ B

analysis of this case will be reported elsewhere (Puel, manuscript in preparation).

RESULTS

The index case is the only child of nonconsanguineous Polish parents living in Germany. In the family, there is no history of recurrent infections or infant deaths. The mother underwent a thorough and detailed physical examination and is healthy, without evidence of increased susceptibility to infections, abnormal dentition, or neurologic, skeletal, or skin abnormalities (normal pigmentation, hair, sweating, and dentition). The patient presented to us at the age of 15 months with recurrent infections and lymphadenitis. His length and weight were in the third percentile (75 cm; 9400 g). He had received complete routine vaccination but had not received BCG vaccination.

At the age of 2 years, *Mycobacterium avium* was identified to cause lymphadenitis for the first time. Tuberculin skin tests had been repeatedly negative, and other DTH responses to *Candida albicans* and tetanus toxoid were not performed. Immunologic work-up at the age of 15 months revealed low IgA (10 mg/dL) and IgG serum levels (135 mg/dL), whereas IgM was markedly elevated (315 mg/dL). At the age of 2 years and 4 months, lymphocyte and lymphocyte subset numbers as well as the mitogen response of lymphocytes were normal (Tables II–IV). PPD response was not performed. Defects in CD40 or CD40 ligand (CD154) expression and autosomal-recessive hyper-IgM were excluded. The boy was put on intravenous immunoglobulin substitution at a dosage of 400 mg/kg body weight, resulting in trough levels of 400 to 600 mg IgG/dL.

At the age of 4 years, lymphadenitis colli recurred. An incomplete neck dissection was performed, and a 4-drug antimycobacterial treatment regimen was given. Subcutaneous IFN- γ was added after it was shown that IFN- γ secretion by stimulated mononuclear cells was markedly reduced (Table IV). IFN- γ was well tolerated except occasional temperatures as high as 39°C within hours after the injection.

At the age of 7 years, the patient developed pulmonary symptoms. Chest x-ray and computerized tomography of the chest were initially normal. However, at the age of 8 years, saccular bronchiectases in the basal segments of both lungs were first documented by computerized tomography of the chest. Within 18 months, repeated cycles of antibiotic treatment led to a slow resolution of the chronic cough.

At the age of 9 years, a skeletal dissemination of mycobacteria into the thoracic and lumbar spine, pelvis, and both femora was suspected by ⁶⁷gallium scan, confirmed by magnetic resonance imaging (MRI). Again, multidrug antimycobacterial therapy was instituted, and 18 months after diagnosis of skeletal dissemination, MRI showed that there was only little residual fatty infiltration left.

At the age of 10 years, several bluish, partly ulcerated skin infiltrates of 1 to 5 cm diameter appeared on the trunk and the extremities while the patient was still on multidrug antimycobacterial therapy (Fig 1). Besides infiltrating lymphocytes, histiocytes, and neutrophils, the skin biopsy showed an epithelioid infiltrate consisting of multinucleated giant cells, strongly suggestive of mycobacterial infiltrates.

At the age of 11 years and 8 months, a hemizygous mutation (110-111insC) in exon 2 of the *NEMO* gene was identified, which causes a frameshift and premature stop codon at position 49. There were no signs of ectodermal dysplasia (EDA), with a normal facial appearance, dentition, and hair (Fig 2) and a normal neurologic examination at that time.

At the age of 12 years and 3 months, the patient was admitted with a history of rapidly increasing pallor and fatigue. In addition to the skin abnormalities described, he had signs of anemia as well as acrocyanosis independent of room temperature. Autoimmune hemolytic anemia was diagnosed on the basis of a marked agglutination of the blood macroscopically, a positive direct Coombs test with demonstration of cold agglutinins and C3d activation on red blood cells, a decreased serum C4 complement (<6 mg/dL), and an increased reticulocyte count (118‰). His hemoglobin had dropped from 9.4 g/L at the last outpatient visit 4 weeks before to 5.1 g/L. There were normal ferritin values and no signs of infectious disease or bleeding. Mycoplasma and EBV infection were excluded by serology and PCR. Antimycobacterial therapy and IFN- γ were discontinued to rule out an adverse effect of one of the antimycobacterial drugs and/or IFN- γ . As his condition rapidly worsened with tachycardia and increasing weakness, prednisone (2 mg/kg) was given, which stabilized his clinical condition promptly, led to disappearance of the acrocyanosis, and increased the hemoglobin to 8.4 g/L within 5 days after admission, when he was discharged. Prednisone was tapered to <0.2 mg/kg within 5 weeks, antimycobacterial therapy was sequentially reintroduced, and his hemoglobin remained stable.

In October 2003, 6 weeks after the severe hemolytic episode, the patient presented with a herpes simplex virus (HSV)–1 encephalitis. Bilateral hyperintense areas mainly

TABLE I. Synopsis of infections in the index patient

Infection	Age	Clinical presentation	Infectious agent	Treatment
Lymphadenitis	15 mo.	Enlargement of cervical, angular, submandibular, and retroauricular lymph nodes	Nothing isolated	Erythromycin
Infections of the upper airways, lymphadenitis	2 y	Cough, rhinitis, enlargement of left cervical, right supraclavicular lymph nodes	<i>Mycobacterium avium</i> (lymph node)	Lymph nodes excised; clarithromycin (15 mg/kg/d), ethambutol (15 mg/kg/d), rifabutin (10mg/kg/d) for 6 mo followed by clarithromycin, rifabutin for 6 mo; residual lymph nodes excised
Lymphadenitis	4 y	Enlargement of cervical, angular, and supraclavicular lymph nodes	Nothing isolated	Incomplete neck dissection, ethambutol, clarithromycin, rifabutin for 3 mo, amikacin 15 mg/kg/d intravenously for 10 d; IFN- γ 50 μ g/m ² /d subcutaneously, 3 days a week continuously
Upper respiratory infections	7 y	Chronic cough without fever	<i>H influenzae</i> , <i>S pneumoniae</i> (sputum)	4 cycles amoxicillin and sulbactam by mouth for 14-21 d within 18 mo, cefuroxime for 4 wk, acetylcysteine, daily physiotherapy
Osteomyelitis	9 y	Night sweats, bone pain, subfebrile temperatures between 38°C and 38.5°C, and loss of weight (1.2 kg in 3 mo)	<i>Mycobacterium avium</i> (bone biopsy, blood)	Multidrug antimycobacterial therapy (ethambutol, clarithromycin, rifabutin at standard dosages, amikacin 15 mg/kg/d for 14 d) repeated every 3 months
Labial herpes infection	10 y	Labial blister	HSV-1 (blister fluid)	Acyclovir orally (3 $\times$ 15 mg/kg/d) for 6 d
Enteritis	11 y	Profuse diarrhea, fever	<i>S enteritidis</i> (stool, blood)	Ciprofloxacin by mouth, 10 d
Encephalitis	12 y	Tremor, weakness of left upper extremity, difficulties to concentrate, general psychomotor slowing.	HSV-1 (cerebrospinal fluid)	Acyclovir 3 $\times$ 20 mg/kg/d, intravenously; IFN- α 3,000,000 U/m ² subcutaneously, 3 times weekly

of the frontal and occipital regions and around the capsula interna were demonstrated by MRI on the same day. Two weeks later, the size of the lesions had progressed in the MRI despite high-dose intravenous acyclovir and IFN- α treatment. His neurologic status deteriorated, and the patient died 6 months later.

DISCUSSION

To our knowledge, we report the first male patient with a *NEMO* mutation without any sign of anhidrotic EDA. There appear to be similar cases in other centers² (Holland et al, manuscript in preparation), reinforcing the general value of our observation. The clinical presentation of male patients with *NEMO* mutations is usually characterized by EDA, a developmental syndrome with absence of sweat glands, sparse scalp hair, and abnormal conical teeth. Although there is some clinical heterogeneity in the

presentation of EDA, the facial appearance is generally typical.¹² Mutations can affect signaling of ectodysplasin (expressed in keratinocytes, hair follicles, sweat glands) through the receptor for ectodysplasin (EDAR) or its associated adaptor protein.¹³ EDA is thought to arise in carriers of *NEMO* mutations because signaling through EDAR requires NF κ B activation. Our case now suggests that EDAR signaling can to some extent stay functional with no clinical consequences depending on the type and location of the mutation in the *NEMO* gene, whereas other mutations clearly lead to ectodermal changes.⁹ Similarly, signaling downstream of RANK and vascular endothelial growth factor 3 appears to be intact in our patient, because there is no osteopetrosis or lymphedema.

Concerning immunodeficiency, the increased susceptibility to mycobacterial infections is a striking feature of the clinical presentation in this case. After exclusion of defects in the IFN- γ loop (eg, normal IFN- γ receptor and IL-12 receptor expression; data not shown) the identification of

TABLE II. Immunologic findings of the patient: humoral immune system

	Age 2 y	Age 4 y	Age 11 y
Immunoglobulins			
IgG	125 (350-1000)*	464 (500-1300)†	447 (700-1400)†
IgG ₁	83	ND	ND†
IgG ₂	19	ND	ND
IgG ₃	17	ND	ND
IgG ₄	1,1	ND	ND
IgA	16 (30-120)	<15 (40-180)	<6 (70-230)
IgM	338 (40-140)	446 (40-180)	582 (40-150)
IgE	<2 IU/mL	ND	<2 IU/mL
Specific antibodies			
Tetanus toxoid	Negative	ND	ND
Diphtheria toxoid	Negative	ND	ND
<i>Haemophilus influenzae</i> B antibodies	1:30 (>1:700)	ND	ND
Pneumococcus antibodies	<1:250 (mean, 1:3552)	ND	ND
Isohemagglutinins, anti-AB, blood group 0	Negative	ND	ND

ND, Not determined.

*Values represent mg/dL unless indicated otherwise. Numbers in parentheses represent the normal age-related range or laboratory normal values for antibody titers.

†On intravenous immunoglobulin substitution.

TABLE III. Immunologic findings of the patient: cellular immune system: major lymphocyte subsets by fluorescence-activated cell sorting analysis

	Age 2 y	Age 4 y	Age 11 y
Leukocytes	16,300/μL	14,300/μL	6200/μL
Lymphocytes	5379 (3600-8900)*	4433 (2300-5400)	1922 (1900-3700)
T cells (CD3 ⁺)	4849 (2100-6200)	2704 (1400-3700)	961 (1200-2600)
CD4 cells	4088 (1300-3400)	2482 (700-2200)	653 (650-1500)
CD8 cells	860 (620-2000)	487 (490-1300)	269 (370-1100)
B cells (CD20 ⁺)	161 (720-2600)	443 (390-1400)	384 (270-860)
Natural killer cells (CD56 ⁺ CD3 ⁻)	215 (180-920)	1019 (130-720)	518 (100-480)
T-cell phenotype			
CD3 ⁺ HLA-DR ⁺	ND	ND	31% (1% to 8%)†
CD4 ⁺ CD45RA ⁺	ND	ND	24% (61%‡)
CD4 ⁺ CD95 ⁺	ND	ND	83% (20%§)
CD4 ⁺ annexin V ⁺	ND	ND	3% (12%§)
CD8 ⁺ CD28 ⁻	ND	ND	68% (32%§)
CD8 ⁺ CD95 ⁺	ND	ND	74% (18%§)
CD8 ⁺ annexin V ⁺	ND	ND	27% (15%§)
B-cell phenotype			
IgD ⁻ CD27 ⁺	ND	ND	0%
IgD ⁺ CD27 ⁻	ND	ND	100%

ND, Not determined.

*Unless indicated otherwise, absolute count/μL; 10th and 90th percentiles in parentheses according to age groups 12-24 mo, 2-6 y, and 6-12 y.¹⁹

†According to Comans-Bitter et al.²⁰

‡Normal median percentage value in parentheses according to age group 7-17 y.²¹

§Laboratory normal values as mean percentage values in parentheses.^{22,23}

a mutation in the *NEMO* gene confirmed previous observations that NFκB activation is a nonredundant part of immunity against mycobacteria. Moreover, there is a humoral immunodeficiency with HIGM phenotype. B cells show a complete lack of memory B cells with an immature IgD⁺CD27⁻ phenotype (Table II), similar to cord blood B cells.¹⁴ Defective immunoglobulin switch in our patient appears to confirm that activation of NFκB is an important part of downstream CD40-mediated signaling.

It seems likely that bronchiectasis and repeated infections with *Haemophilus influenzae* and *Streptococcus pneumoniae* were complications of the mucosal, humoral (secretory) immunoglobulin deficiency. It is not clear, however, to what extent the defective NFκB activation in cells other than B cells (eg, CD40 expressing alveolar macrophages)¹⁵ may have facilitated the development of these pulmonary sequelae in our case. *Salmonella enteritidis* infection has been described in 1 patient with OL-EDA-

TABLE IV. Immunologic findings of the patient: functional assays of the cellular immune system: response of mononuclear cells to mitogen (72 h) and tetanus toxoid stimulation (96 h); intracellular calcium mobilization and IFN- γ production of mononuclear cells

	Age 2 y	Age 4 y	Age 11 y
Mitogen/antigen stimulation			
PHA (1 μ g/mL)	38.500 (44.420)*	41.700 (68.800)	38.000 (44.000)
Pokeweed mitogen (1 μ g/mL)	39.190 (27.860)	21.300 (33.500)	38.500 (42.000)
OKT3 (1 μ g/mL)	19.170 (42.470)	9.200 (42.800)	18.500 (27.000)
SAC (1:1000)	2.870 (10.920)	21900 (7.800)	2.500 (10.000)
Tetanus toxoid	0 (7.110)	100 (2400)	0 (7.000)
Intracellular calcium mobilization			
Before stimulation	ND	ND	53 (60)†
PHA	ND	ND	223 (294)
Anti-CD3	ND	ND	62 (68)
Anti-CD3 ⁺ goat antimouse Ig crosslink	ND	ND	154 (203)
IFN- γ production			
Medium	ND	11 (19 $\pm$ 11)‡	ND
IL-2	ND	17 (229 $\pm$ 175)	ND
IL-2 + anti-CD3	ND	170 (1001 $\pm$ 660)	ND

ND, Not determined.

*Standard 3H thymidine incorporation assay as described.²⁴ Counts per minute of the patient and of a control performed on the same day (in parentheses) are expressed as mean of triple measurements. Medium values were subtracted in all measurements. Medium values of the patient and the healthy controls were below 500 counts per minute in all 3 experiments.

†Measurement of free cytosolic calcium by using the fluorescence indicator fura-2 as described.²⁵ Values represent nmol/L intracellular calcium of the patient and of a control performed on the same day (in parentheses).

‡pg/mL; ELISA at age 6 y.



FIG 1. Partly ulcerated skin infiltrate of 5-cm diameter on the right lower extremity at age 10 years.



FIG 2. Index case with normal facial features.

ID.⁹ In our case, it appeared to be related to travel and responded appropriately to conventional antibiotic therapy. The fatal outcome of HSV-1 encephalitis despite prompt treatment with high-dose acyclovir may indicate an increased susceptibility to HSV infections as part of the immunodeficiency. There is a second well-documented case of HSV encephalitis in signal transducer and activator 1 deficiency.¹⁶ The death of 1 patient with OL-EDA-ID was associated with another viral infection, adenovirus gastroenteritis.⁹ Compromised *in vitro* natural killer cell function has been demonstrated in 3 cases with NEMO deficiency, which may be associated with impaired antiviral immunity.¹¹ Moreover, our case was complicated

by severe intravascular autoimmune Coombs-positive hemolytic anemia caused by formation of IgM cold agglutinins. Hemolysis led to splenectomy in a patient with EDA-OL patient, but the etiology of the hemolysis was not described in detail.⁶ Autoimmune phenomena are not uncommon in immunodeficient patients and have been observed in patients with defective CD40-mediated signaling.¹⁷

The diagnosis of a patient with this form of X-linked immunodeficiency may be difficult and delayed. The initial presentation of a child with a NEMO mutation may be subtle with lymphadenitis only, which is a common problem in the pediatrician's office and is sometimes

caused by atypical mycobacteria. The initial work-up of the immune system may be completely normal (especially at younger age, when the hyper-IgM phenotype may not yet be apparent). The differential diagnosis of increased susceptibility to mycobacterial infections includes many inborn and acquired defects of the IFN- γ -IL-12 axis, which can be distinguished by the absence of a hyper-IgM phenotype.¹⁸ Other genotype-phenotype correlations may exist, in which increased susceptibility to mycobacteria may present later, and children may come to attention with clinical signs of a B-cell deficiency and laboratory findings of a switching defect (elevated IgM; low IgG, IgA, and IgE). The classic causes of a HIGM (mutations in CD40L, CD40, activation-induced cytidine deaminase, uracil-DNA glycosylase genes) must be excluded before analysis of NEMO is performed. If there is both HIGM and infection with atypical mycobacteria or BCGitis, analysis of the NEMO gene is mandatory, because classic cases of HIGM syndromes are not associated with atypical mycobacteria or BCG infections.

Clearly, only limited conclusions can be drawn from a single case regarding treatment and prognosis. Supplementation with intravenous IgG is mandatory because of the B-cell switching defect. Long-term multidrug antimycobacterial therapy combined with surgical procedures is necessary for recurrent lymphadenitis, because immunity against mycobacteria is severely impaired. We could demonstrate that a 4-drug regimen given over a period of 18 months can bring skeletal dissemination into near-complete remission (by MRI). We were not able to discontinue the multidrug antimycobacterial therapy afterward, because there was no response regarding the cutaneous infiltrates. Clearly, such an approach needed close monitoring for side effects (eg, optic neuritis, hepatotoxicity, and so forth). We chose not to put the patient on permanent prophylactic antibiotics to avoid side effects and the development of resistant strains. Substitution with IFN- γ appeared reasonable because of the reduced production of IFN- γ . The therapeutic effect of IFN- γ in our case was not clear, because cutaneous and skeletal dissemination occurred despite IFN- γ substitution. On the other hand, we cannot exclude that there would have been a diminished response to multidrug antimycobacterial therapy had there been no IFN- γ substitution. The treatment of viral infections needs to be aggressive, because increased susceptibility to viral infections cannot be excluded. Once viral disease (eg, HSV, cytomegalovirus) has occurred, a secondary prophylaxis may be useful (eg, acyclovir, ganciclovir). In the case of encephalitis, addition of cytokines such as IFN- γ and IFN- α did not show an effect. There is evidence that IL-2 may correct the killing defect in NEMO deficient children *in vitro*, but as of yet, there is no experience in using IL-2 *in vivo*.¹¹ Finally, our patient could have been a candidate for transplantation with hematopoietic stem cells. However, conditioning with immunosuppression (probably even with a nonmyeloablative regimen) bears a high risk of disseminated mycobacteriosis, and fatal organ toxicity has been observed in a child with OL-EDA-

ID.¹⁰ In summary, we present the case of a NEMO-deficient child with presence of mycobacterial disease as well as fatal encephalitis yet without anhidrotic EDA.

We thank the patient and his family and deeply respect how they coped with this fatal disease.

After our manuscript was accepted for publication, we were informed that a similar report would appear in the Journal.²⁶

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