The presentation and natural history of immunodeficiency caused by nuclear factor κB essential modulator mutation

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Background: An increasing number of rare genetic defects are associated with immunodeficiency and impaired ability to activate gene transcription through nuclear factor (NF) κB. Hypomorphic mutations in the NFκB essential modulator (NEMO) impair NFκB function and are linked to both immunodeficiency and ectodermal dysplasia (ED), as well as susceptibility to atypical mycobacterial infections.
Objective: We sought to investigate the clinical and immunologic natural history of patients with *NEMO* mutation with immunodeficiency (NEMO-ID).
Methods: Patients with severe bacterial infection and ED or unexplained mycobacterial sensitivity were evaluated for *NEMO* mutation. Laboratory investigations and clinical data were retrospectively and prospectively accumulated and reviewed.
Results: We have given a diagnosis of NEMO-ID to 7 boys; 6 had ED, and 5 had gene mutations in the 10th exon of *NEMO*. Our resulting estimated incidence of NEMO-ID is 1:250,000 live male births. All patients had serious pyogenic bacterial illnesses early in life, and the median age of first infection was 8.1 months. Most boys had mycobacterial disease (median age, 84 months), and a minority had herpesviral infections. Initial immunologic assessments showed hypogammaglobulinemia (median IgG, 170 mg/dL) with variable IgM (median, 41 mg/dL) and IgA (median, 143 mg/dL) levels. Two patients had increased IgM levels, and 5 had increased IgA levels. All patients evaluated had normal lymphocyte subsets with impaired proliferative responses, specific antibody production, and natural killer cell function. Two patients died from complications of mycobacterial disease (ages 21 and 33 months).
Conclusion: NEMO-ID is a combined immunodeficiency with early susceptibility to pyogenic bacteria and later susceptibility to mycobacterial infection. Specific features of particular

NEMO mutations in these patients provide insight into the role of this gene in immune function. (J Allergy Clin Immunol 2004;113:725-33.)

A growing family of diseases are the result of gene mutations that impair nuclear factor (NF) κB activation.[1,2] The classic model of NFκB activation posits that NFκB family members are maintained in the cell cytoplasm bound to an inhibitor of NFκB (IκB) that prevents entry to the nucleus to activate transcription.[3] During cell activation, signals are generated that result in the assembly of the IκB kinase complex (IKK), which phosphorylates IκB. Phospho-IκB is ubiquitinated and degraded, freeing NFκB to dimerize and translocate to the nucleus.

One phenotype that highlights this pathway clinically is ectodermal dysplasia (ED). ED is a syndrome characterized by dental abnormalities, eccrine sweat gland dysgenesis, characteristic facies, pale wrinkled skin, and fine sparse hair. An important role for NFκB activation in the pathogenesis of ED was appreciated after a majority of cases were linked to mutations of the *ED1* gene on the X chromosome encoding the TNF superfamily member ectodysplasin-A.[4,5] It was subsequently found that mutations in the genes encoding the ectodysplasin-A receptor (a TNF receptor superfamily member),[6] as well as its associated death domain,[7] result in autosomally inherited forms of ED. These findings suggested that NFκB activation is required for effective signaling and ectodermal development mediated by this TNF superfamily system. A more direct link between ectodermal development and NFκB was made on observation of mutant mice rendered deficient for the α subunit of the IKK complex. These mice had a variety of cutaneous defects reminiscent of ED and an inability to activate NFκB in the skin.[8,9]

A human example of defective IKK function resulting in an ectodermal phenotype was initially found in women with incontinentia pigmenti. Incontinentia pigmenti is a disease characterized by dermal scarring and hyperpigmentation that has been associated with large deletions or frameshift mutations in one allele of the NFκB essential modulator (NEMO; also known as IKK-γ) gene, alternatively referred to as *IKBKG*, present on the X chromosome.[10] This mutant *NEMO* allele is nonfunctional, and

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Abbreviations used
CYLD: Cylindromatosis tumor suppressor
ED: Ectodermal dysplasia
ED-ID: ED with immunodeficiency
IκB: Inhibitor of NFκB
IKK: IκB kinase
MAC: *Mycobacterium avium* intracellulare
NEMO: NFκB essential modulator
NEMO-ID: *NEMO* mutation with immunodeficiency
NF: Nuclear factor
NK: Natural killer
TLR: Toll-like receptor

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male offspring who inherit it die in utero because some NFκB activation is essential for development. Although NEMO does not possess a catalytic function, it serves as a scaffold for other IKK members, it is an important link to upstream regulators, and it is clearly required for NFκB activation.

A subset of boys with ED was also known to have immunodeficiency (ED with immunodeficiency [ED-ID]).[11] Although historical accounts of these patients are variable, they include both cellular and humoral immune abnormalities.[11-15] Because NFκB function is required by many immunoreceptors, as well as for ectodermal development, several groups have studied NFκB function in boys with ED-ID.[16-21] Hypomorphic mutations in the *NEMO* gene and resulting impairment of NFκB activation were linked with this phenotype.[19-21] Most boys had mutations affecting the 10th and final exon of *NEMO*, which encodes a zinc finger domain,[16-21] and a minority had point mutations elsewhere in the gene.[20,21]

Immunologic characteristics described for boys with hypomorphic *NEMO* mutations include hypogamma-globulinemia and specific antibody deficiency.[16-22] In vitro studies demonstrated impairments in CD40-mediated B-cell activation, isotype class switching, natural killer (NK) cell cytotoxicity, response to LPS stimulation, and production of TNF and IL-12.[19-21] These defects appear to result in specific infectious susceptibilities because patients having ED-ID and a *NEMO* mutation are extraordinarily vulnerable to atypical mycobacteria. However, the natural history and variability of presentation of hypomorphic *NEMO* mutations have not been described. In this work we present a 20-year experience with 7 patients having hypomorphic *NEMO* mutations with immunodeficiency.

METHODS

Patients

Our patients all presented for evaluation of immunodeficiency to Children's Hospital Boston between 1984 and 2002. All studies were performed with informed parental consent−child assent and were approved by the Children's Hospital Committee on Clinical Investigation.

Brief clinical presentations, *NEMO* mutation, and some immunologic characteristics of patients 1 to 3 have been described in a previous publication and correspond to patients 1 to 3 in that report.[21]

Additional investigations of NFκB activation in patient 1 have also been performed.[23]

Patient 4 was born at term and was healthy until his 10th month, when meningitis associated with a febrile seizure developed. Lumbar puncture yielded purulent cerebrospinal fluid, and culture grew *Streptococcus pneumoniae*. His only significant prior medical history was a lichenoid dermatitis noted at 2 months that responded to topical corticosteroids. He had defective NFκB activation, as determined on the basis of impaired CD40-induced B-cell function, and reduced nuclear localization of NFκB, as demonstrated by using an electrophoretic mobility shift assay.[23]

Patient 5 had *Haemophilus influenzae* sepsis and was initially given a diagnosis of relative IgG2 and IgG3 subclass deficiencies. He perspired normally and never demonstrated characteristics of ED. The details of his phenotype, genotype, and NFκB activation are reported elsewhere (manuscript in preparation).

Patients 6 and 7 were half brothers born to the same mother. Their clinical presentation and gene mutation were previously described, and they were designated family 4 III-1 (patient 6) and 4 III-2 (patient 7).[16] These boys presumably did not have the hyper-IgM syndrome caused by CD40 ligand deficiency because patient 7 had normal expression of CD154 (performed as previously described).[24]

NEMO sequence analysis

Genomic DNA and cDNA were prepared from lysed patient leukocytes. Genomic DNA was analyzed first, and if a potential mutation was identified, cDNA was sequenced.[19,21] cDNA was evaluated because the presence of a *NEMO* pseudogene can lead to invalid conclusions if analyses are based on genomic DNA alone.[25] The primers and approach for sequencing exons 4, 9 (manuscript in preparation), and 10 were as previously described.[19,21] The resulting sequences were compared initially with the consensus in GenBank (AN-AJ271718) and with those on 40 or more X chromosomes from healthy individuals.[21]

Immunologic assays

Serum immunoglobulin concentrations (determined by means of nephelometry) leukocyte enumeration, nitroblue tetrazolium reduction, and total hemolytic complement were measured in the Children's Hospital Clinical Laboratories and compared with laboratory-specific age-related normal values. Lymphocyte subset analyses and mitogen- and antigen-induced proliferation were performed as previously described.[26] The number and percentage of patient lymphocytes in various subsets were compared with published age-related normal values.[27] NK cell cytotoxicity was evaluated on the basis of 51Cr-release from radiolabeled K562 erythroleukemia cells, and results were expressed as K562 lytic units, as previously described.[28]

Statistical analyses

NEMO-ID incidence rates were approximated by using the US Government census data for the catchment area, including Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont, and were obtained from http://eire.census.gov/popest/data/states/ST-EST2002-ASRO-01.php. Immunologic data are presented as means ± SDs. When indicated, data sets were compared by using the Student *t* test.

RESULTS

Diagnosis of ED-ID or NEMO-ID

A diagnosis of NEMO-ID was considered on the basis of severe infection with characteristics of ED in patients 1 to 4, 6, and 7, or recurrent infection and atypical mycobacterial disease in patient 5. Before evaluation for
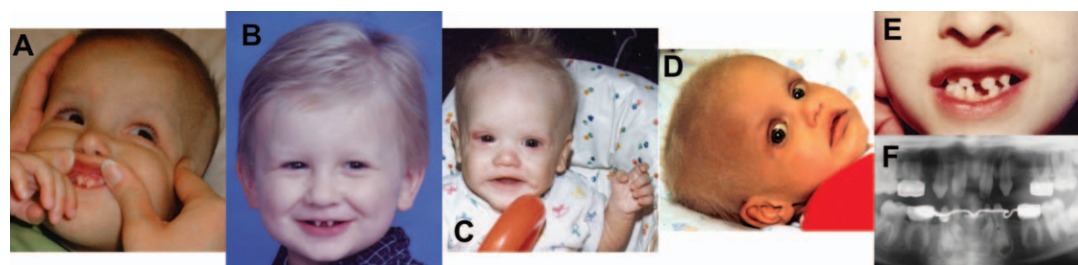
FIG 1. Facies of boys with ED-ID and a *NEMO* mutation. Patients 1 **(A)**, 4 **(B)**, 6 **(C)**, and 7 **(D)** demonstrate many typical features of ED, including hypotrichosis, pale skin, depressed nasal bridge, frontal bossing, conical incisors, and oligodontia. Dental features are prominent, as seen in a magnified oral view of patient 3 **(E)** and a dental radiograph from patient 2 **(F)**.
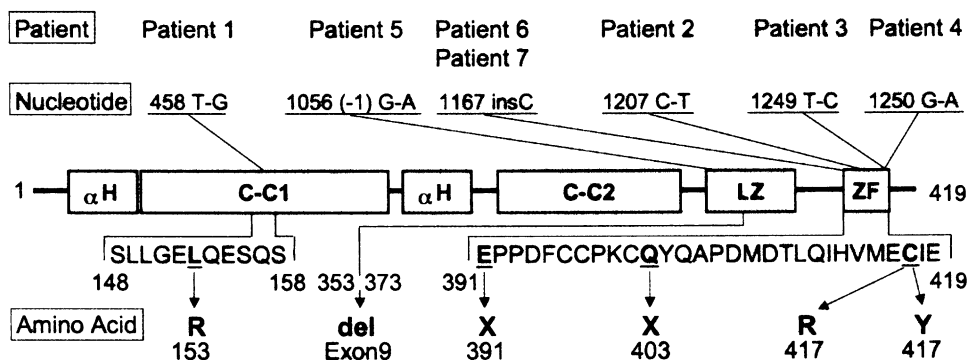
FIG 2. Summary of *NEMO* mutations in 7 patients with NEMO-ID. *NEMO* sequence alterations are listed above a schematic of the NEMO protein, with a line directed to the approximate location in the protein that is affected. *αH,* Alpha helix; *C-C1,* first coiled-coil domain; *C-C2,* second coiled-coil domain; *LZ,* leucine zipper; *ZF,* zinc finger. The nucleotide change is listed for the cDNA. The amino acid sequence for affected regions is enlarged beneath the appropriate regions of the NEMO schematic, and predicted residue alterations are demonstrated with an *arrow.* In the case of patient 5, the mutation affected exon 9, which is deleted *(del)* because of splice site alteration occurring upstream of nucleotide position 1056.

a *NEMO* mutation, extensive immunologic investigations effectively excluded known genetic and acquired causes of immunodeficiency. The characteristics of ED for diagnosis are described[11] and included the following: hypohidrosis; conical or peg-shaped teeth with oligodontia; hypotrichosis; frontal bossing; pale skin relative to parental pigmentation; and depressed nasal bridge. Patients 1 to 4, 6, and 7 had all the aforementioned criteria (Fig 1). Patient 5 did not demonstrate any of these characteristics. In the patients with ED, this diagnosis was entertained after the appearance of abnormal dentition. In our patients the first teeth to erupt typically were abnormally spaced conical upper incisors (Fig 1). The mean age of tooth eruption was 16.8 ± 5 months.

ID was diagnosed before ED in all patients, and laboratory assessments for ID were performed at a median age of 4 months (range, 1-73 months). ID was considered in patients 1 and 3 to 7 because of severe infection (bacterial sepsis in patients 1, 3, 6, and 7 and severe pneumonia in patient 5). ID was pursued in patient 2 because of unexplained recurrent fevers starting in his first month of life. Because most boys in this series were given a diagnosis of ID before the discovery of *NEMO* mutation as a cause of ED-ID,[16] the age at which genetic diagnosis was estab-

lished was not indicative of clinical suspicion for the disorder. The diagnosis was conferred postmortem in patients 6 and 7. The specifics of *NEMO* genetic analysis were previously reported for all patients (outlined in the Methods section), except for patient 4. He had a G-to-A substitution at position 1250 in his *NEMO* cDNA, which results in a predicted substitution of Y for C at position 417 in the zinc finger. This alteration and a summary of the other patient's mutations are presented in Fig 2.

On the basis of this number of molecular genetic diagnoses among tabulated births from within the designated catchment area of our institution over the duration of our study period (see the Methods section; 1 patient was excluded because of his origin from outside the area), the incidence of *NEMO* mutations resulting in NEMO-ID is not less than 1 in 250,000 live male births.

The mothers of all patients were also evaluated. The mothers of patients 1, 4, 6, and 7 were carriers, whereas those of patients 2, 3, and 5 were not. Only the mother of patient 1 had features reminiscent of ED, including oligodontia (4 missing secondary teeth), with one conical tooth, some alopecia, and large areas of hyperpigmentation on her thighs. The maternal grandmother of patient 1 was also a carrier and had multiple sclerosis in her third

TABLE I. Disease susceptibility in children with a *NEMO* mutation having ED-ID or NEMO-ID

Patient no.	Pyogenic bacteria			Mycobacteria			Other pathogens		Comorbidity
	Disease	Age (mo)	Organism	Disease	Age (mo)	Pathogen	Pathogen	Disease	Disease
1	Sepsis	0.1	*Listeria* species	None			CMV MCV*	Sepsis colitis molluscum	Colitis
2	Sepsis	35.3	*Pneumococcus* species	Cutaneous disseminated	84	*M avium*	HSV HPV*	Pharyngitis flat warts	Arthritis
3	Sepsis	8.1	*Klebsiella* species	Osteomyelitis	168	*M abcessus*	*Giardia* MCV*	Enteritis molluscum	
4	Meningitis	9.7	*Pneumococcus* species	None					
5	Sepsis	60.9	*Haemophillus* (HIB)	Cutaneous	166	*M bovis*			
6	Sepsis	0.1	*Pseudomonas* species	Disseminated	14	*M avium*			
7	Sepsis	1.9	*Klebsiella* species	Disseminated	22	*M avium*	*P carinii*	Pneumonia	
Mean		16.6 ± 23.0			91 ± 75				

Means ± SDs are shown.

MCV, Molluscum contagiosum virus; *HPV,* human papilloma virus; *HIB, Haemophillus influenzae* type B.

*A disease presumed, but not proved, to be caused by the pathogen listed.

decade but did not have findings of ED. The mother of patient 4 had been given a diagnosis of juvenile rheumatoid arthritis at age 6 years and Behçet disease at age 11 years.

Infections

Boys with NEMO-ID had life-threatening bacterial illness (either sepsis or meningitis) at a median age of 8.1 months (range, 0.1-60.9 months; Table I). In 2 patients these infections were caused by pathogens for which the children had been immunized (patients 4 and 5). In 3 patients (1, 6, and 7) the infections occurred in the perinatal period. In the boys with later onset of life-threatening bacterial illness (patients 2 and 5), there was an earlier history of presumed bacterial pneumonia with radiographic evidence of pulmonary infiltrate (patient 2 at 11 months and patient 5 at 36 months). Thus a consistent feature of NEMO-ID was a susceptibility to severe pyogenic bacterial infections in early infancy or childhood.

Diseases caused by other pathogens were also prominent, and 5 boys were infected with atypical mycobacteria (Table I). The median age at which signs and symptoms ultimately attributed to mycobacterial infection were recognized was 84 months (range, 14-168 months). *Mycobacterium avium* intracellulare (MAC) was diagnosed by means of culture in 3 patients, and *Mycobacterium abcessus* and *Mycobacterium bovis* were diagnosed in one each. Only patient 5 was able to successfully stop multidrug antimycobacterial chemotherapies without relapse of his disease. Disseminated MAC infection was the cause of death in the 2 patients who died (patient 6 at 21 months and patient 7 at 33 months) before knowledge of the NEMO-ID diagnosis. An autopsy performed on patient 7 demonstrated miliary nodules in the spleen, liver, and

lungs, as well as acid fast bacilli in the liver, spleen, lungs, adrenal glands, and lymph nodes.

Several boys also had severe nonbacterial infections. Patient 1 had CMV sepsis and 2 subsequent episodes of biopsy-proved CMV colitis that all responded to ganciclovir treatment. He received 6 months of subcutaneous IL-2 therapy (1×10^6 units 3 times per week) during which he was free of CMV disease, and he was ultimately maintained on valganciclovir prophylaxis. Patient 2 had herpes simplex virus stomatitis and pharyngitis that required acyclovir therapy, and the patient has not had recurrence. Both patients 1 and 3 had chronic molluscum contagiosum, and patient 2 had numerous flat warts. Only 2 patients had notable protozoal diseases. Patient 3 had *Giardia lamblia* enteritis, and patient 6 had *Pneumocystis carinii* pneumonia (after he was given a diagnosis of MAC). Fungal infections occurred in patients 3 and 7. Patient 3 had *Candida albicans* sepsis, which was probably a complication of indwelling catheters, and patient 2 had prolonged thrush.

Comorbid conditions

Patient 1 had persistent diarrhea, feeding intolerance, failure to thrive, and perianal fistulas. Endoscopic evaluations demonstrated inflammation and ulceration of the esophagus, stomach, ileocecum, and colon. Biopsies showed nonspecific inflammation without granulomas. Inflammatory bowel disease was suspected after associated infectious agents were not found, and he was successfully treated with oral 6-mercaptopurine and corticosteroids. Patient 2 had recurrent large joint arthritides that interfered with his activity and partially responded to nonsteroidal anti-inflammatory therapy. Although a diagnosis of atopy had been entertained in most boys, only one had detectable IgE antibodies
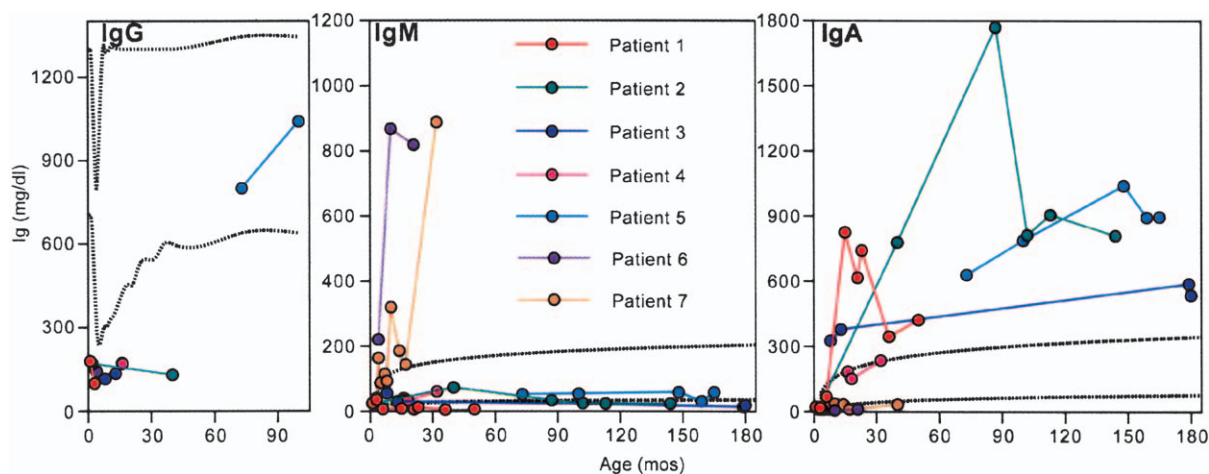
FIG 3. Serum immunoglobulin concentrations in patients with NEMO-ID. Serum IgG *(left panel)*, IgM *(middle panel)*, and IgA *(right panel)* concentrations were measured at presentation and over time. Each individual patient is denoted by a specific color, as shown in the legend in the *middle panel*. The *dashed black lines* mark the 5th and 95th percentile limits for age. IgG levels were plotted until the patients were started on intravenous immunoglobulin therapy and not thereafter.

(10 IU/mL). Skin prick test responses or results of assays to detect specific IgE antibodies were negative in the 4 patients who had such testing.

Immunologic findings

Initial immunologic assessments in all but one boy (patient 5) showed hypogammaglobulinemia (Fig 3), and the median serum IgG concentration in this subgroup was 162 mg/dL (range, 116-179 mg/dL). The distribution of IgG subclasses was assessed in 3 patients (1, 4, and 5), who all had IgG2 levels of 10% or less of total IgG levels (patient 5 also had undetectable IgG3 levels). The median serum IgM concentration was 41 mg/dL (range, 12-221 mg/dL), and only patient 6 presented with a level greater than the 95th percentile for age. The median serum IgA concentration was 21 mg/dL (range, 8-630 mg/dL), with 3 patients having a level greater than the 95th percentile for age. The evolution of immunoglobulin isotype concentrations over time conformed to one of 2 distinct patterns: the first was increased IgM levels to greater than the 95th percentile for age and IgA levels of less than the 5th percentile for age (patients 6 and 7), and the second was increased IgA levels to greater than the 95th percentile for age with low or normal IgM levels (patients 1-5, Fig 3). Specific antibody production was assessed in response to tetanus immunization. Six patients had received at least 2 tetanus toxoid vaccinations; only 3 had detectable tetanus-specific IgG, and only 1 had a level of greater than 0.2 IU/mL (Fig 4, *A*). Interestingly, both patients with C417 substitutions in *NEMO* failed to make any specific IgG. Intravenous immunoglobulin therapy was initiated in all patients at a median age of 18 months (range, 4-148 months).

Early in life, leukocyte counts were persistently increased with normal differentials, and the median WBC count at the initial immunologic evaluation was 13,210 cells/μL (range, 10,970-55,570 cells/μL). WBC counts returned to within normal ranges by 30 months of age. Although WBC differentials were generally normal, patient 2 experienced transient eosinophilia (24%) of unknown cause. All other boys had 2% or less eosinophils. Lymphocyte populations were typically within age-specific limits (Fig 5). The relative proportions of CD3^{+} T cells, CD4^{+} T cells, and CD8^{+} T cells of total lymphocytes was maintained within normal ranges over time (Fig 6). Exceptions were patient 2, who had low percentages of CD3^{+} and CD4^{+} T cells by his eighth year, and patient 7, who had a low percentage of CD8^{+} T cells before his death at 33 months.

Lymphocyte proliferation in response to PHA and pokeweed mitogen was normal in all patients (except pokeweed mitogen in patient 3) but decreased to concanavalin-A in patients 1, 2, and 3 (Fig 4, *B*). In contrast, tetanus or diphtheria antigen−induced proliferation resulted in stimulation indices of less than 4 in most cases (Fig 4, *C*) compared with greater than 10 in the majority of healthy control subjects. Only patient 5 had stimulation indices of greater than 4 in response to both antigens. For patients 1, 3, 4, and 6, anti-CD3−induced proliferation was variable (median stimulation index, 16.4; range, 3.3-96.0).

NK cell cytotoxic activity was decreased in all living patients in our series relative to control donors (median lytic units of 41 and range of 11-84 for patients vs 251 and 203-898, respectively, for control subjects; $P = .01$; Fig 4, *D*). Complement function was normal in the 6 patients tested. The results of nitroblue tetrazolium reduction assays were normal in 5 patients.

DISCUSSION

Although patients having NEMO-ID were in part originally identified on the basis of their susceptibility to
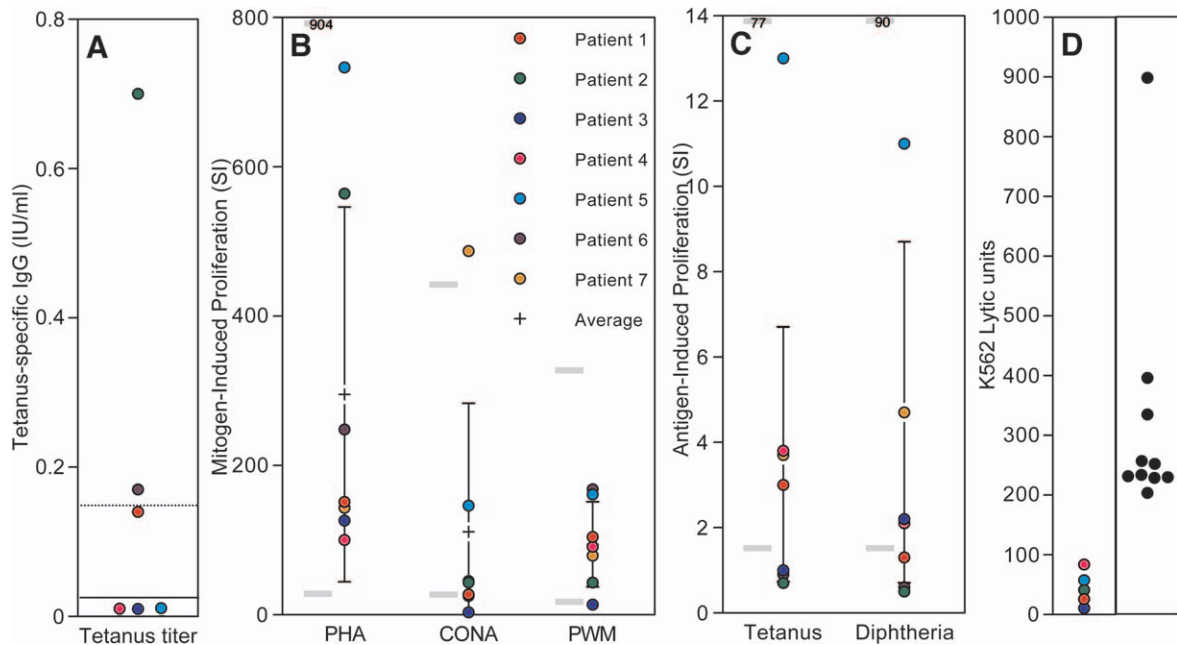
FIG 4. Lymphocyte and humoral immune function in patients with NEMO-ID. **A,** Serum tetanus-specific IgG level is shown relative to the level considered to be protective *(dashed line)*. Patients with undetectable levels are shown beneath the *solid line*. Initial assessments of PHA-, pokeweed mitogen *(PMA)*−, and concanavalin-A− *(CONA)* **(B)**, as well as tetanus- and diphtheria-induced proliferation **(C)**, are shown for each patient and expressed as stimulation indices. **D,** NK cell cytotoxicity was measured in patients who were alive at the time of this report as K562 lytic units. Patient NK cell activity *(left, colored circles)* is compared with that in 10 healthy control donors *(right, black circles)*. All patient values are represented with colored circles according to the legend, and values are shown as means ± SDs. The age-normalized 5th and 95th percentiles for the parameters provided in Fig 4, *B* and *C,* are approximated with *gray bars*, and when the 95th percentile value is greater than the graph scale, the value is shown within the bar.

mycobacterial infections, a hallmark of the boys described here was severe pyogenic bacterial infections early in life. This feature highlights the underlying pathophysiology of the gene mutation. Aside from its role in adaptive immunoreceptor signaling, NFκB activation is essential for function of innate immunoreceptors. In particular, toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns, all use NFκB signaling pathways.[29] The bacterial cell-wall component LPS binds to TLR-4 and was incapable of inducing a TNF response in a boy with a *NEMO* mutation.[20] Thus one possible explanation for the susceptibility to infection with pyogenic bacteria soon after birth in patients with NEMO-ID is that TLR signaling and innate immunity is defective. Impaired TLR signaling might also explain the increased occurrence of mycobacterial infections because certain TLRs recognize mycobacterial components. Interestingly, maternal IgG did not protect these boys from bacterial illness in the newborn period, emphasizing the critical role of innate immunoreceptor function. These characteristics suggest that severe pyogenic bacterial illnesses should prompt consideration of NFκB activation disorders. In this regard, other genetic defects that impair the nuclear translocation of NFκB after ligation of TLRs are associated with pyogenic bacterial infections as well.[30,31]

NEMO-ID has both clinical and immunologic heterogeneity. Although it is impossible to eliminate a contribu-

tion of an individual's genetic background, disease variability is also at least partly a result of the hypomorphic nature of the *NEMO* mutations. The most severe clinical courses in this series were found in the boys with E391X alterations (patients 6 and 7), resulting in a greater than 50% truncation of the 10th exon. Interestingly, when the NEMO truncation involved less than 50% of the 10th exon (Q403X), the phenotype was compatible with longer-term survival (patient 2). This suggests a role for specific regions of the 10th exon in binding other regulatory proteins. Mutations that alter the charge of exon 10 are also informative. Replacement of the cysteine at 417 with a more basic residue (patients 3 and 4) appears to have significant effects on the function of the protein. These boys consistently have impaired class switching and demonstrate the most severe B-cell phenotype.[19,21] It is unclear whether this alteration affects the direct binding of other regulatory molecules to the NEMO C-terminus or the higher-order structure of the folded protein.

If the primary effect of exon 10 *NEMO* mutations is on the interaction of other regulatory proteins with this region, a noteworthy candidate is the cylindromatosis tumor suppressor (CYLD). CYLD is a deubiquinase that binds to the C-terminal 39 amino acids of *NEMO* and serves as a negative regulator of NFκB activation.[32] Mutations of *CYLD* result in increased activation of NFκB and are associated with the cutaneous tumor syndrome familial
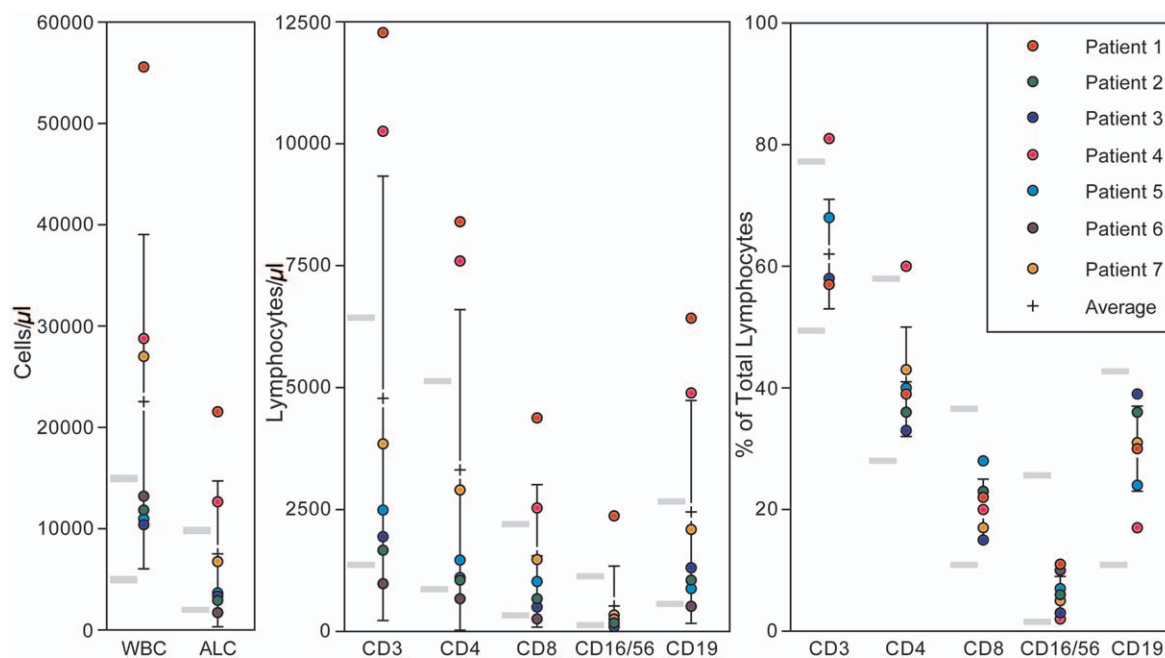
FIG 5. Leukocyte counts and lymphocyte subsets in patients with NEMO-ID. Numbers of total WBCs and the absolute lymphocyte count *(ALC; left panel)*, as well as specific lymphocyte subsets *(middle panel)* per milliliter of peripheral blood, were determined at the time of initial immunologic evaluation. Lymphocyte subpopulations as a proportion of total lymphocytes are also shown *(right panel)*. The lymphocyte subtypes evaluated are presented on the x axis and include CD3+ T cells, CD3+/CD4+ T cells, CD8+/CD3+ T cells, CD3−/CD16+/CD56+ NK cells and CD3−/CD19+ B cells. Individual patient values are represented by colored circles as per the legend, and values are shown as means ± SDs. The age-normalized 5th and 95th percentiles for the parameters provided are approximated with *gray bars*.

cylindromatosis.[33] Importantly, the C417R mutant of NEMO failed to bind to CYLD.[34] Thus it is likely that a variety of alterations or truncations of the extreme C-terminus of NEMO might affect the affinity it has for CYLD. CYLD also binds the TNF receptor−associated factor 2, which is an upstream activator of IKK.[32,34] Thus in addition to its deubiquinating function, CLYD might serve an adapter function and approximate molecules required for IKK activation.

We also describe the natural history of 2 boys having mutations in *NEMO* outside of the 10th exon. These defects appear to be significantly less common. Including this series, there have been 22 families described as having NEMO-ID. Seventy-three percent of mutations affect exon 10, and 44% of these alter position C417 (changing it to arginine in 57%, phenylalanine in 29%, and tyrosine in 14%). Gene defects found outside of exon 10 associated with NEMO-ID affecting each of exons 4 to 9 have now been described. All of these boys had ED, except our patient 5, who had an altered ninth exon (manuscript in preparation). In our patients and those that have been reported elsewhere,[14,15] non−exon 10 mutations were all associated with specific antibody deficiency. Importantly, the majority had increased IgA levels (except for exon 6 mutation), and all had low-to-normal IgM levels. Our in vitro data demonstrated that B cells from these boys could produce IgE after CD40 ligation compared with those with C417 substitutions, which could not.[21] Thus there are notable potential genotype-phenotype correlations in boys with NEMO-ID that warrant further study and will likely provide insight into the function of the IKK complex.

Our series highlights several immunologic patterns that were previously underappreciated. Most strikingly, 5 of 6 mutations studied were associated with significantly increased IgA levels (Fig 3). Defects in B-cell costimulation typically result in increased IgM and decreased IgA levels.[35] Although this pattern is clearly seen in a subset of boys with exon 10 *NEMO* mutations, in other patients the presence of extremely high levels of IgA with low IgG levels might challenge some traditional notions of class switching. This might represent a particular feature of a hypomorphic NEMO that can still allow certain signals to occur. We underscore, however, the clinical relevance of this abnormality and suggest that NEMO-ID should be considered in boys with increased IgA levels who have severe pyogenic bacterial infections or mycobacterial infections early in life. We also recommend caution in the use of the term ''hyper IgM'' to describe the immunologic phenotype associated with NEMO-ID.

We have extended our previous finding of decreased NK cell cytotoxic activity[21] to all of our living patients (Fig 4, *D*). Thus NEMO-ID joins a growing list of human genetic defects that impair NK cell function.[36] Infectious susceptibilities common to these disorders suggest an important role for NK cells in host defense. Importantly, in a subset of patients, antibody-dependent cellular
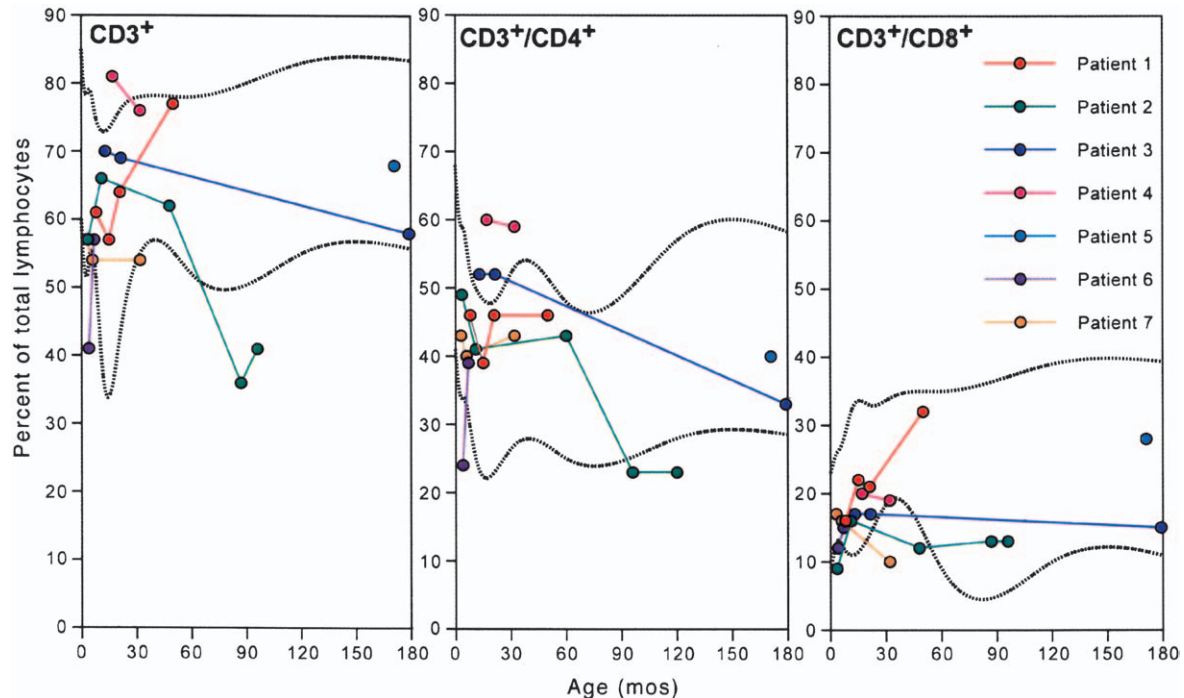
FIG 6. Alterations in lymphocyte subsets over time in patients with NEMO-ID. Total CD3$^+$ T cells *(left)*, CD3$^+$/CD4$^+$ T cells, and CD3$^+$/CD8$^+$ T cells are shown as a percentage of total lymphocytes over time. Individual patient values are represented by colored circles connected with a line of the same color as per the legend. The *lower* and *upper dashed lines* show age-related limits of the 5th and 95th percentile, respectively.

cytotoxicity was evaluated and was normal,[21] highlighting a dichotomy in NK cell−NK cell signaling and activities. The defect in NEMO-ID therefore implies a critical involvement of NEMO and NFκB signaling pathways in specific NK cell functions.

Finally, it is critical to consider issues specific to the clinical care of boys with NEMO-ID mutation. Appropriate genetic diagnosis and genetic counseling are essential, and *NEMO* carrier testing should be offered to the patient's mother and sisters, as well as maternal aunts if appropriate. Boys with a *NEMO* mutation and evidence of impaired specific antibody production should be treated with intravenous immunoglobulin. MAC prophylaxis should be considered because of the high incidence of this infection. *Pneumocystis carinii* pneumonia in one of our patients also leads us to suggest an awareness for this diagnosis and a consideration of specific prophylaxis. Viral disease caused by herpesviruses should be treated aggressively, and a chemoprophylaxis regimen should also be considered. At this time, it is premature to comment on stem cell transplantation because there is limited experience. To our knowledge, only one patient with NEMO-ID has undergone successful stem cell transplantation. The boy was conditioned with busulfan and cytoxan, and the donor was a human leukocyte antigen−identical sibling (Dr D. Pietryga, personal communication). Because boys with NEMO-ID are given diagnoses earlier in life, having had less infectious complications, it will be important to provide directed clinical care in an attempt to improve outcome.

In summary, NEMO-ID is characterized by specific infectious susceptibilities and immunologic impairments and has opened doors to the clinical consideration of a new facet of innate immune defense, highlighting the importance of innate immunity. These observations also suggest that defects in innate immunity probably are responsible for a portion of the infant mortality rate and that targeted diagnosis of these disorders in families having concerning histories will be fruitful.

Note added in proof

After the acceptance of this manuscript, another protein, Bc110, has been shown to act on NEMO and require an intact NEMO zinc finger for function (Zhou et al. Nature 2004;427:167-71). It is likely that this interaction is impaired in certain NEMO-ID patients and participates in the mechanism underlying the immunodeficiency.

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