

The Presentation and Natural History of Immunodeficiency due to NF κ B Essential Modulator Mutation.

Jordan S. Orange MD, PhD,^{a*} Ashish Jain MD,^b Zuhair K. Ballas, MD,^c Lynda C. Schneider, MD,^a
Raif S. Geha, MD,^a Francisco A. Bonilla, MD, PhD^a

^aDepartment of Immunology, Children's Hospital, Boston, MA, ^bNational Institutes of Health, Bethesda, MD, ^cUniversity of Iowa, Iowa City, IA.

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Please address correspondence to: Francisco A. Bonilla, MD, PhD, Division of Immunology, Children's Hospital Boston, 300 Longwood Av, Boston MA 02115.

*Current address: Division of Immunology, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, 3615 Civic Center Boulevard, Philadelphia, PA 19104.

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Abstract

Background: An increasing number of rare genetic defects are associated with immunodeficiency and impaired ability to activate gene transcription via NF κ B. Hypomorphic mutations in the NF κ B essential modulator (NEMO) impair NF κ B function and are linked to both immunodeficiency and ectodermal dysplasia (ED) as well as susceptibility to atypical mycobacterial infections.

Objective: To investigate the clinical and immunological natural history of patients with NEMO mutation and immunodeficiency (NEMO-ID).

Methods: Patients with recurrent 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 diagnosed 7 boys with NEMO-ID; 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 mos. Most boys developed mycobacterial disease (median age=84mos) and a minority had herpesviral infections. Initial immunological assessments showed hypogammaglobulinemia (median IgG=170mg/dl) with variable IgM (median=41mg/dl) and IgA (median=143mg/dl). Two patients developed hyper-IgM and five developed hyper-IgA. All patients evaluated had normal lymphocyte subsets with impaired proliferative responses,

specific-antibody production and NK cell function. Two patients died from complications of mycobacterial disease (ages 21 and 33mos).

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.

Key Words: NEMO, primary immunodeficiency, NF κ B, innate immunity, ectodermal dysplasia, hypogammaglobulinemia, mycobacteria, NK cells, combined immunodeficiency.

Abbreviations:

CMV - Cytomegalovirus

ED – Ectodermal dysplasia

ED-ID – ED with ID

ID – Immunodeficiency

I κ B – Inhibitor of NF κ B

IKK – I κ B kinase

MAC – Mycobacterium avium intracellulare

NBT – Nitroblue tetrazolium

NEMO – NF κ B essential modulator

NEMO-ID – NEMO mutation with ID

NF κ B – Nuclear factor κ B

NK – Natural killer

TLR – Toll-like receptor

Introduction

A growing family of diseases are the result of gene mutations that impair NF κ B activation.^{1, 2} The classic model of NF κ B activation posits that NF κ B family members are maintained in the cell cytoplasm bound to an inhibitor of NF κ B (I κ B) that prevents entry to the nucleus to activate transcription.³ During cell activation, signals are generated that result in the assembly of the I κ B kinase complex (IKK) which phosphorylates I κ B. Phospho-I κ B is ubiquitinated and degraded, freeing NF κ B 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 NF κ B 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 (EDA)^{4, 5}. It was subsequently found that mutations in the genes encoding the EDA receptor (a TNF receptor superfamily member)⁶ as well as its associated death domain⁷ result in autosomally-inherited forms of ED. These findings suggested that NF κ B activation is required for effective signaling and ectodermal development mediated by this TNF superfamily system. A more direct link between ectodermal development and NF κ B was made upon 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 NF κ B 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 (IP). IP is a disease characterized by dermal scarring and hyperpigmentation that has been associated with large deletions or frameshift mutations in one allele of NEMO (also known as IKK- γ) present on the X chromosome.¹⁰ This

mutant NEMO allele is non-functional and male offspring who inherit it die *in utero* since some NF κ B activation is essential for development. Although NEMO does not possess catalytic function, it serves as a scaffold for other IKK members, it is an important link to upstream regulators, and is clearly required for NF κ B activation.

A subset of boys with ED were also known to have immunodeficiency (ectodermal dysplasia with immunodeficiency; ED-ID).¹¹ Although historical accounts of these patients are variable, they include both cellular and humoral immune abnormalities.¹¹⁻¹⁵ Since NF κ B function is required by many immunoreceptors as well as for ectodermal development several groups have studied NF κ B function in boys with ED-ID.¹⁶⁻²¹ Hypomorphic mutations in the NEMO gene and resulting impairment of NF κ B activation were linked with this phenotype.¹⁹⁻²¹ Most boys had mutations affecting the 10th and final exon of NEMO that 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 hypogammaglobulinemia, and specific antibody deficiency.¹⁶⁻²² *In vitro* studies demonstrated impairments in CD40-mediated B cell activation, isotype class switching, 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, as 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 approximately a 20yr experience with 7 patients having hypomorphic NEMO mutations and immunodeficiency (NEMO-ID).

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 immunological characteristics of patients 1-3 have been described in a previous publication and correspond to patients 1-3 in that report.²¹ Additional investigations of NF κ B activation in patient 1 have also been performed.²³

Patient 4 was born at term and was healthy until his 10th month when he developed meningitis associated with a febrile seizure. Lumbar puncture was purulent and culture grew *S. Pneumoniae* (a serotype contained within the heptavalent pneumococcal conjugate vaccine). His only significant prior medical history was a lichenoid dermatitis noted at 2mos that responded to topical corticosteroids. He had defective NF κ B activation determined by impaired CD40-induced B cell function, and reduced nuclear localization of NF κ B demonstrated by electrophoretic mobility shift assay.²³

Patient 5 was initially diagnosed with recurrent infection and relative IgG2 and IgG3 subclass deficiencies. He perspires normally and never demonstrated characteristics of ED. The details of his phenotype, genotype and NF κ B 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 where they were designated family 4 III-1 (patient 6) and 4 III-2 (patient 7).¹⁶

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 then cDNA was sequenced.^{19, 21} cDNA was evaluated because the presence of a NEMO pseudogene can lead to invalid conclusions if analyses are based upon genomic DNA alone.²⁴ The primers and approach for sequencing exons 4, 9 (manuscript in preparation) and 10 were as described.^{19, 21} The resulting sequences were compared initially to the consensus in GenBank ([AN-AJ271718](#)) and to those on ≥ 40 X chromosomes from healthy individuals.

Immunologic assays

Serum immunoglobulin concentrations (determined by nephelometry) leukocyte enumeration, nitroblue tetrazolium (NBT) reduction, and total hemolytic complement were measured in the Children's Hospital Boston Clinical Laboratories and compared to laboratory-specific age-related normal values. Lymphocyte subset analyses and mitogen and antigen-induced proliferation were performed as described.²⁵ Patient lymphocyte subsets were compared with published age-related normal values.²⁶ NK cell cytotoxicity was evaluated by ⁵¹Cr-release assay against K562 erythroleukemia cells and results expressed as K562 lytic units as described.²⁷

Statistical analyses

NEMO-ID incidence rates were approximated using the U.S. Government census data for the catchment area including ME, MA, NH, RI and VT and was obtained from <http://eire.census.gov/popest/data/states/ST-EST2002-ASRO-01.php>. Immunologic data are presented as mean \pm S.D. When indicated, data sets were distinguished using Student's *t* test.

Results

Diagnosis of ED-ID or NEMO-ID

A diagnosis of NEMO-ID was considered due to recurrent or severe infection with characteristics of ED in patients 1-4, 6 and 7, or recurrent infection and atypical mycobacterial disease in patient 5. Prior to evaluation for a NEMO mutation, extensive immunologic investigations effectively excluded known genetic causes of immunodeficiency. The characteristics of ED for diagnosis are described,¹¹ and included the following: (A) hypohidrosis; (B) conical or peg-shaped teeth with oligodontia; (C) hypotrichosis; (D) frontal bossing; (E) pale skin relative to parental pigmentation; (F) depressed nasal bridge. Patients 1-4, 6 and 7 had all aforementioned criteria (Figure 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 abnormal spaced conical upper incisors (Figure 1). The mean age of tooth eruption was 16.8 ± 5 mos.

ID was diagnosed prior to ED in all patients and laboratory assessments for ID were performed at a median age of 4 mos (range 1-73 mos). ID was considered in patients 1 and 3-7 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. As most boys in this series were diagnosed with ID prior to the discovery of NEMO mutation as a cause of ED-ID¹⁶, the age at which genetic diagnosis was established was not indicative of clinical suspicion for the disorder. The diagnosis was conferred post-mortem in patients 6 and 7. The specifics of NEMO genetic analysis were previously reported for all patients (outlined in the *Methods*), 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 Figure 2.

Based upon this number of molecular genetic diagnoses among tabulated births over the duration of our study period from within the designated catchment area of our institution (see Methods, one patient was excluded due to his origin from outside this 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, while 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 3rd decade, but did not have findings of ED. The mother of patient 4 had been diagnosed with juvenile rheumatoid arthritis at age 6 and Bechet's disease at age 11.

Infections

Boys with NEMO-ID had life threatening bacterial illness (either sepsis or meningitis) at a median age of 8.1 mos (Range=0.1-60.9; Table 1). In two cases, these infections were caused by pathogens for which the children had been immunized (patients 4 and 5). In three 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 recurrent sinopulmonary infections including presumed bacterial pneumonia (patient 2 at 11 mos and patient 5 at 36 mos). All boys except patient 4 required multiple courses of both enteral and parenteral antibiotics for sinopulmonary bacterial infections. Thus, a consistent and early feature of NEMO mutation was a susceptibility to pyogenic bacterial infections.

Diseases caused by other pathogens were also prominent, and five boys were infected with atypical mycobacteria (Table 1). The median age at which signs and symptoms ultimately attributed to mycobacterial infection were recognized was 84mos (range=14-168mos). *M. avium intracellulare* (MAC) was diagnosed by culture in three patients and *M. abscessus* and *M. bovis*, in one each. Only patient 5 was able to successfully stop multi-drug anti-mycobacterial chemotherapies without relapse of his disease. Disseminated MAC infection was the cause of death in the two patients who expired (patient 6 at 21mos and patient 7 at 33mos) prior to knowledge of the NEMO-ID diagnosis.

Several boys also had severe non-bacterial infections. Patient 1 had cytomegalovirus (CMV) sepsis and two subsequent episodes of biopsy-proven CMV colitis that all responded to gancyclovir treatment. He received 6mos of subcutaneous IL-2 therapy (1×10^6 units 3x/wk) during which he was free of CMV disease, and he was ultimately maintained on valgancyclovir prophylaxis. Patient 2 had herpes simplex virus stomatitis and pharyngitis that required acyclovir therapy, and has not had recurrence. Both patients 1 and 3 had chronic molluscum contagiosum, and patient 2 had numerous flat warts. Only two patients had notable protozoal diseases. Patient 3 had *Giardia lamblia* enteritis, and patient 6 had *Pneumocystis carinii* pneumonia (after he was diagnosed with 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. His symptoms and pathology were successfully treated with oral 6-mercaptopurine and corticosteroids. Patient 2 had recurrent large joint arthritides that interfered with his activity and partially responded to non-steroidal anti-inflammatory therapy. Although a diagnosis of atopy had been entertained in most boys at some point, only one had detectable IgE (10IU/ml). Skin prick tests or assays to detect specific IgE were negative in the four who had such testing.

Immunologic findings

Initial immunologic assessments in all but one boy (patient 5) showed hypogammaglobulinemia (Figure 3) and the median serum IgG concentration in this subgroup was 162mg/dL(range=116-179). The distribution of IgG subclasses was assessed in three patients (1,4, and 5) who all had IgG2 \leq 10% of total IgG (Patient 5 also had undetectable IgG3). Median serum IgM concentration was 41mg/dL (range=12-221) and only patient 6 presented with a level $>95^{\text{th}}$ percentile for age. Median serum IgA concentration was 21mg/dL (range=8-630) with 3 patients having a level $>95^{\text{th}}$ percentile for age. The evolution of immunoglobulin isotype concentrations over time conformed to one of two distinct patterns: the first was elevated IgM $>95^{\text{th}}$ percentile for age and IgA $<5^{\text{th}}$ percentile for age (patients 6 and 7) and the second was elevated IgA $>95^{\text{th}}$ percentile for age with low or normal IgM (patients 1-5, Figure 3). Specific antibody production was assessed in response to tetanus immunization. Six patients had received at least two tetanus toxoid vaccinations; only three had detectable tetanus-specific IgG and only one had a level above 0.2 IU/ml (Figure 4A). Interestingly, both patients with C417 substitutions in NEMO failed to make any specific IgG. IVIG therapy was initiated in all patients at a median age of 18mos (range=4-148mos).

Early in life, leukocyte counts were persistently elevated with normal differentials, and the median WBC count at the initial immunologic evaluation was 13,210 cells/mm³ (range 10,970-55,570). 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 $\leq 2\%$ eosinophils. Lymphocyte populations were typically within age-specific limits (Figure 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 (Figure 6). Exceptions were patient 2 who had low percentages of CD3⁺ and CD4⁺ T cells by his 8th year, and patient 7 who had a low percentage of CD8⁺ T cells prior to his death at 33 months. Lymphocyte proliferation in response to phytohemagglutinin and pokeweed mitogen was normal in all patients (except PWM in patient 3), but depressed to concanavalin-A in patients 1, 2, and 3 (Figure 4B). In contrast, tetanus or diphtheria antigen-induced proliferation resulted in stimulation indices < 4 in most cases (Figure 4C) compared to > 10 in the majority of healthy controls. Only patient 5 had stimulation indices > 4 in response to both antigens. For patients 1, 3, 4 and 6 anti-CD3-induced proliferation was variable (median stimulation index was 16.4, range=3.3-96.0)

NK cell cytotoxic activity was depressed in all living patients in our series relative to control donors (median lytic units=41, range=11-84 for patients vs 251, range=203-898 for controls; $p=0.01$, Figure 4D). Complement function was normal in six patients tested. NBT reduction assays were normal in five patients.

Discussion

Although patients having NEMO-ID were in part originally identified due to their susceptibility to 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, NF κ B activation is essential for function of innate immunoreceptors. In particular, toll-like receptors (TLR) which recognize pathogen associated molecular patterns, all utilize NF κ B signaling pathways.²⁸ The bacterial cell wall component lipopolysaccharide 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 NEMO-ID is that TLR signaling and innate immunity is defective. Impaired TLR signaling may also explain the increased occurrence of mycobacterial infections, as certain TLR 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 NF κ B activation disorders. In this regard, other genetic defects that impair the nuclear translocation of NF κ B after ligation of TLR are associated with pyogenic bacterial infections as well.^{29, 30}

NEMO-ID has both clinical and immunologic heterogeneity. Although it is impossible to eliminate a contribution 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 >50% truncation of the 10th exon. Interestingly, when the NEMO truncation affected <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 if 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 upon the interaction of other regulatory proteins with this region, a noteworthy candidate is the cylindromatosis tumor suppressor (CYLD). CYLD is a de-ubiquinase that binds to the C-terminal 39 amino acids of NEMO and serves as a negative regulator of NF κ B activation.³¹ Mutations of CYLD result in increased activation of NF κ B and are associated with the cutaneous tumor syndrome Familial Cylindromatosis.³² Importantly, the C417R mutant of NEMO failed to bind to CYLD.³³ Thus it is likely that a variety of alterations or truncations of the extreme C-terminus of NEMO may affect the affinity it has for CYLD. CYLD also binds the TNF receptor associated factor 2 (TRAF-2), which is an upstream activator of IKK.^{31, 33} Thus, in addition to its de-ubiquinating function, CLYD may serve an adapter function and approximate molecules required for IKK activation.

We also describe the natural history of two 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 having NEMO-ID. 73% 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 4-9 have now been described. All of these boys had ED, except our patient 5 who had an altered 9th 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 elevated IgA (except for exon 6 mutation) and all had low-normal IgM. Our in vitro data demonstrated that B cells from these boys could produce IgE after CD40 ligation compared to those with C417 substitutions who 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, five out of six mutations studied were associated with significant hyper-IgA (Figure 3). Defects in B cell costimulation typically result in elevated IgM and decreased IgA.³⁴ 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, may challenge some traditional notions of class switching. This may 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 hyper-IgA who have severe pyogenic bacterial infections or mycobacterial infections early in life.

Another interesting feature of our patients was early leukocytosis (Figure 5). Several boys were evaluated for leukocyte adhesion deficiency (LAD) and had normal expression of CD18. Although, patient 1 had delayed separation of the umbilical cord remnant (removed at 8wks), all patients had evidence of purulent infections. One explanation for the leukocytosis is sub-clinical chronic infections. An alternative explanation relates to activation of NF κ B after ligation of β 2 integrins.³⁵ Intact integrin-mediated signaling may be required in addition to integrin-mediated adhesion for appropriate leukocyte homing. Defective activation of NF κ B after integrin ligation, therefore, may lead to a partial LAD phenotype in boys with NEMO-ID.

We have extended our previous finding of depressed NK cell cytotoxic activity³⁶ to all of our living patients (Figure 4D). Thus, NEMO-ID joins a growing list of human genetic defects that impair NK cell function³⁶. Infectious susceptibilities common to these disorders suggest an important role for NK cells in host defense. NK cell defects in NEMO-ID also imply a critical involvement of NEMO and NF κ B signaling pathways in NK cell function.

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 IVIG. MAC prophylaxis should be considered due to the high incidence of this infection. *Pneumocystis* pneumonia in one of our patients also leads us to suggest specific prophylaxis, especially in boys with low T cell counts or severely impaired lymphocyte proliferation. Viral disease caused by herpesviruses should be treated aggressively and a chemoprophylaxis regimen considered. At this time, it is premature to comment on stem cell transplantation as 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). As boys with NEMO-ID are diagnosed 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.

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Figure Legends

Figure 1: *Facies of boys with ED-ID and a NEMO mutation.* Patient 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 x-ray from patient 2 (F)

Figure 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. 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 demonstrated with an arrow. In the case of patient 5, the mutation affected exon 9, which is deleted (denoted as “del”) due to splice site alteration occurring upstream of nucleotide position 1056.

Figure 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 IVIG therapy and not thereafter.

Figure 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

phytohemagglutinin (PHA)-, pokeweed mitogen (PMA)- and concanavalin-A- (B) as well as tetanus- and diphtheria-induced proliferation (C) are shown for each patient 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 to that in 10 healthy control donors (right, black circles). All patient values are represented with colored circles according to the legend and means shown (+) \pm S.D.

Figure 5: *Leukocyte counts and lymphocyte subsets in patients with NEMO-ID.* Numbers of total WBCs and lymphocytes (left panel) as well as specific lymphocyte subsets (middle panel) per ml 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 means shown (+) \pm S.D. The age-normalized 5th and 95th percentiles for the parameters provided are approximated with gray bars.

Figure 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.

Table I – Disease susceptibility in children with a NEMO mutation having ED-ID or NEMO-ID.

Patient	Pyogenic bacteria			Mycobacteria			Other Pathogens		Comorbidity
	Disease	Age (mos)	Organism	Disease	Age (mos)	Pathogen	Pathogen	Disease	Disease
1	Sepsis	0.1	Listeria	none			CMV MCV*	Sepsis Colitis Molluscum	Colitis
2	Sepsis	35.3	Pneumococcus	Cutaneous disseminated	84	M. Avium	HSV HPV*	Pharyngitis Flat warts	Arthritis
3	Sepsis	8.1	Klebsiella	osteomyelitis	168	M. Abcessus	Giardia MCV*	Enteritis Molluscum	
4	Meningitis	9.7	Pneumococcus	none					
5	Sepsis	60.9	Hemophilus (HIB)	cutaneous	166	M. Bovis			
6	Sepsis	0.1	Pseudomonas	disseminated	14	M. Avium			
7	Sepsis	1.9	Klebsiella	disseminated	22	M. Avium	P. carinii	Pneumonia	
Mean		16.6±23.0			91±75				

Means are shown ± S.D. An asterisk denotes a disease presumed, but not proven, to be caused by the pathogen listed.

HIB-Hemophilus influenzae type B, CMV-cytomegalovirus, MCV-Moluscum contagiosum virus, HPV-Human papilloma virus

Figure 1

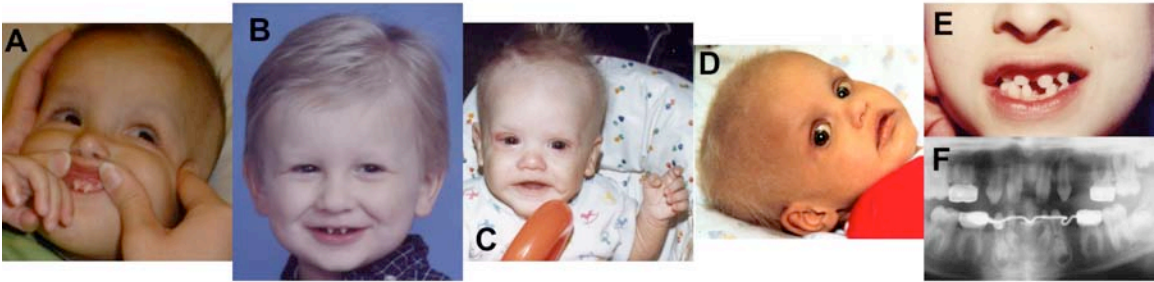


Figure 2

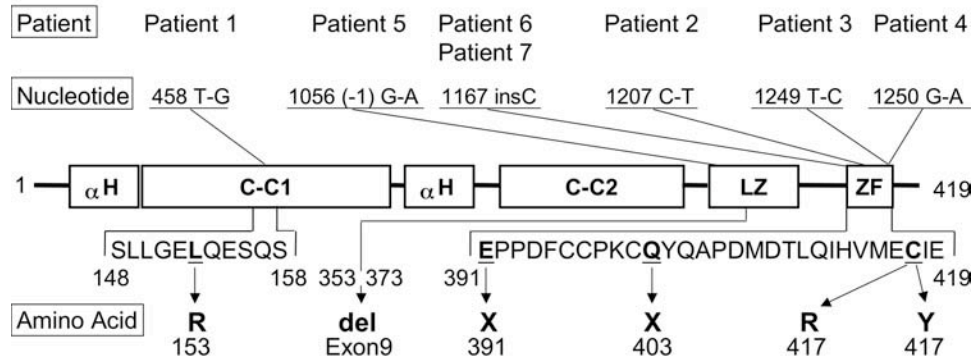


Figure 3

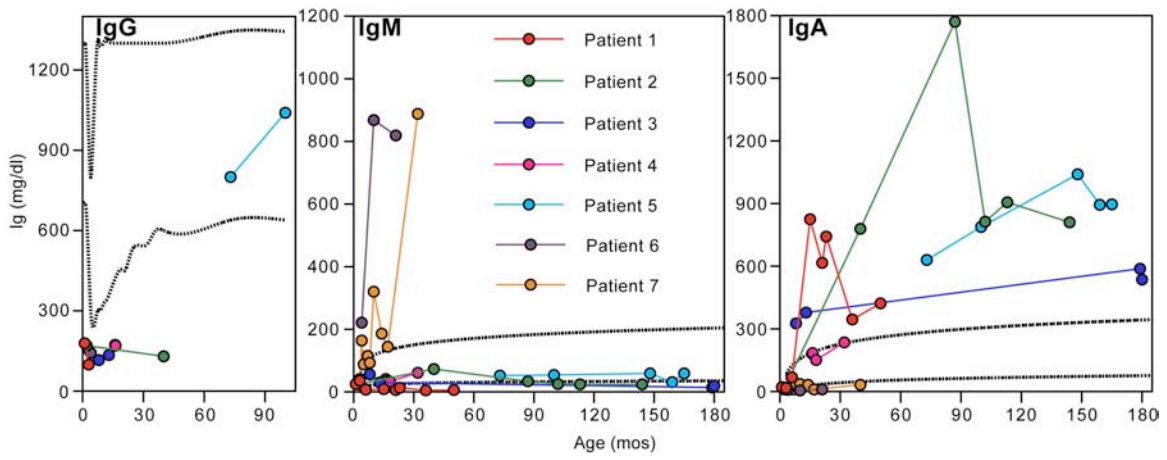


Figure 4

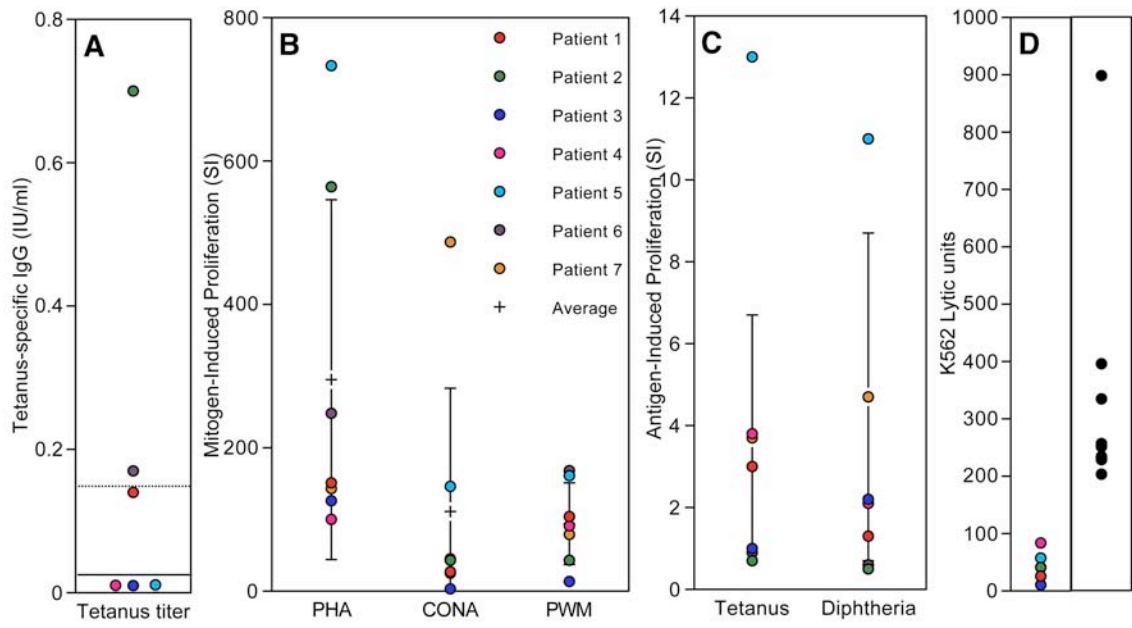


Figure 5

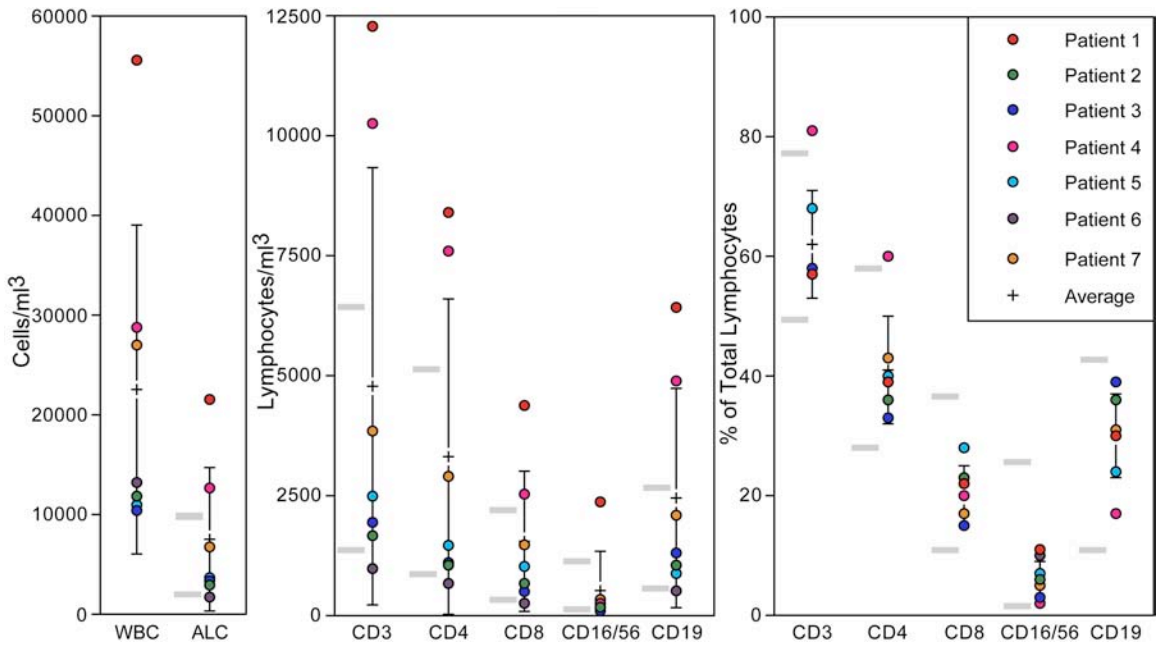


Figure 6

