The range of defects associated with nuclear factor κB essential modulator

Gulbu Uzel

Purpose of review

Impaired ability to signal and activate specific gene transcription through nuclear factor κB (NFκB) has been directly linked to immunodeficiency. Hypomorphic mutations in the gene encoding NFκB essential modulator (NEMO), located on the X chromosome, impair NFκB function and lead to ectodermal dysplasia with immunodeficiency (ED-ID) with increased susceptibility to pyogenic bacteria, viruses and nonpathogenic mycobacterial infections. This is due to impaired, but not abolished, response to a variety of stimuli including Toll-like receptor agonists. Alternatively, loss-of-function (amorphic) mutations in the same gene lead to incontinentia pigmenti. The purpose of this review is to explore the range of immunologic defects associated with mutations in NEMO, a key regulatory molecule in the NFκB pathway.

Recent findings

In addition to the discovery of X-linked recessive hypomorphic mutations in NEMO as the cause of anhidrotic ED-ID, autosomal-dominant hypermorphic mutations in inhibitor of NFκB (IκB) α have been described recently. In addition, a better understanding of genotype–phenotype correlation in ED-ID patients is evolving.

Summary

ED-ID is a combined, variable but profound immunodeficiency characterized by susceptibility to pyogenic bacteria and mycobacterial infection. Understanding the features of particular NEMO mutations will provide insight into the role of this gene and will help define the crucial role of the function and regulation of NFκB in the immune response.

Keywords

ectodermal dysplasia, immunodeficiency, NEMO, NFκB, nonpathogenic mycobacterial infection, nuclear factor, nuclear factor κB essential modulator

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Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Correspondence to Gulbu Uzel, MD, Laboratory of Clinical Infectious Diseases, 9000 Rockville Pike, Bldg 10 CRC B3-4141 MSC 1684, Bethesda, MD 20892-1684, USA
Tel: +1 301 451 9035; fax: +1 301-480-4507; e-mail: guzel@mail.nih.gov

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Abbreviations

ED-ID	ectodermal dysplasia with immunodeficiency
IκB	inhibitor of nuclear factor κB
IKK	inhibitor of nuclear factor κB kinase
NEMO	nuclear factor κB essential modulator
NFκB	nuclear factor κB
RANK	receptor activator of nuclear factor κB
TLR	Toll-like receptor
TNF	tumor necrosis factor
XL-EDA-ID	X-linked anhidrotic ectodermal dysplasia with immunodeficiency

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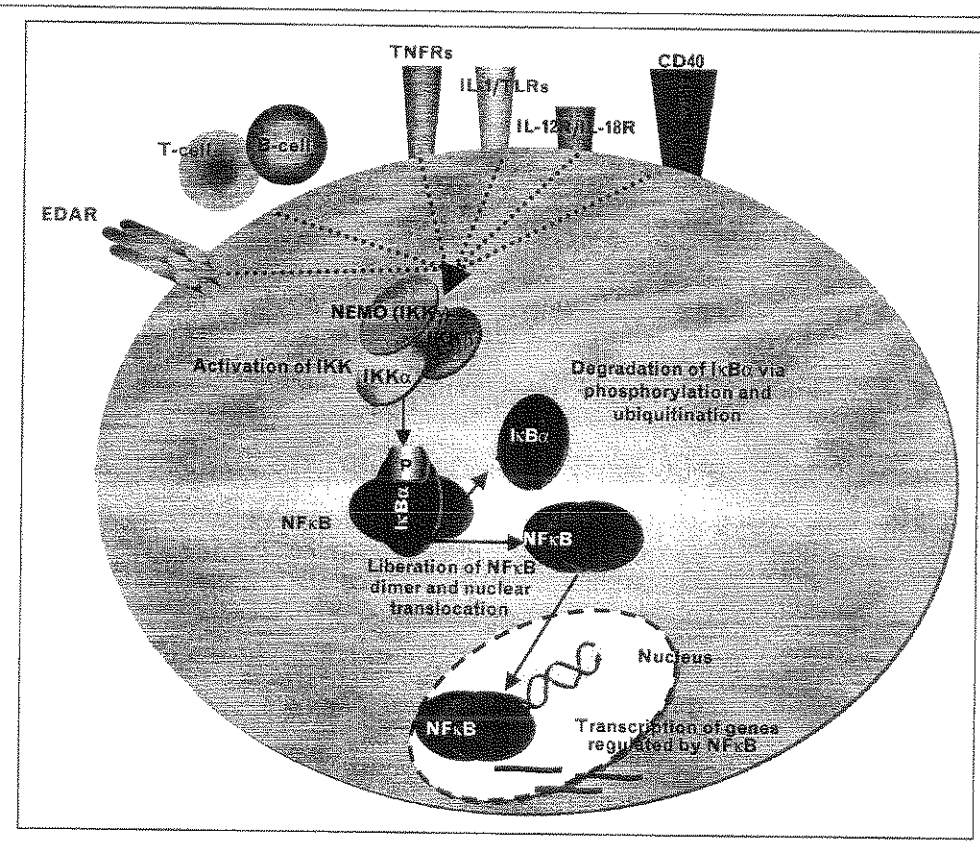
Introduction

Nuclear factor κB (NFκB) has attracted scientific attention because of its unusual regulation, the wide variety of stimuli that activate it and the diverse genes and biological responses that it controls [1,2]. NFκB is a heterogeneous collection of dimers that is expressed ubiquitously. Its interaction with inhibitor of NFκB (IκB) regulates NFκB cytoplasmic retention and function and keeps NFκB inactive in the cytoplasm [3]. The IκB family of proteins includes IκBα (the first one cloned, and also the best characterized), IκBβ, IκBε, IκBγ, Bci-3, p100 and p105 [4,5••]. The IκB kinase (IKK) complex regulates IκB phosphorylation by promoting its ubiquitination and proteosome degradation, and in this way liberates NFκB. The IKK is composed of three subunits: IKKα and IKKβ serve as the two catalytic components, whereas NFκB essential modulator (NEMO; IKKγ) is the structural scaffold that supports the IKK complex without any catalytic function [6,7]. Thus NEMO is essential for the formation of a functional IKK complex (Fig. 1). Once free of its regulatory subunit, NFκB enters the nucleus to initiate transcription of a variety of genes. NFκB-regulated genes range from those involved in immunity, inflammation, apoptosis, adhesion and cell growth [7,8].

Nuclear factor κB essential modulator-knockout mice

Male NEMO-knockout mice (*nemo*$^{-/-}$) devoid of NFκB activity die from liver apoptosis around day 13. In heterozygous female mice (*nemo*$^{+/-}$) transient dermatosis is seen at birth, characterized by patchy skin lesions, massive granulocyte infiltration, hyperproliferation and apoptosis of keratinocytes [9,10]. These findings are very similar to what has been observed in humans with incontinentia pigmenti [11]. Hyperpigmentation, which is the

Figure 1. NFκB signaling cascade.



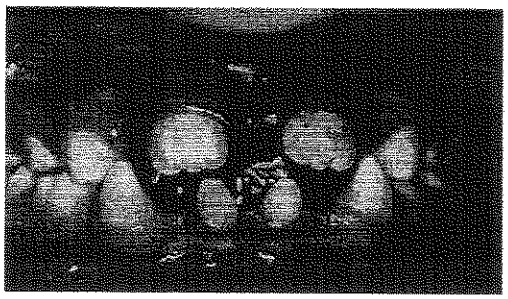
EDAR, ectodysplasin A receptor; IκB, inhibitory κB; IKK, inhibitory κB kinase; IL-R, interleukin receptor; NEMO, nuclear factor κB essential modulator; NFκB, nuclear factor κB; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor.

hallmark of human incontinentia pigmenti, is also seen in skin sections from *nemo*$^{+/-}$ mice [2]. In contrast to wild-type animals, the basal-layer keratinocytes of *nemo*$^{+/-}$ mice are in loose contact with each other, forming filopodia that extend into the dilated intercellular spaces. An explanation for the nature of the skin pathology observed in these female mice and incontinentia pigmenti patients involves the following processes: initially, NEMO cells hyperproliferate because of a lack of NFκB activation and this is followed by death of some of these cells inducing an inflammatory reaction. This involves chemokine and cytokine release by neighboring healthy cells, leading to an increased death rate of mutant cells [11]. Severe skewing of X-chromosome inactivation in the lymphocyte compartment in *nemo*$^{+/-}$ mice suggests that lymphocytes carrying an active copy of the mutated X chromosome are specifically eliminated after birth. However, in contrast to what is known in humans, female *nemo*$^{+/-}$ mice exhibit early lethality depending on the murine genetic background [9,10].

Nuclear factor κB essential modulator defects: an immunodeficiency with unusual features

Three independent investigations have identified the relationship between mutations in NEMO and immunodeficiency. Zonana *et al.* [12] described four families in which males presented with variable degrees of X-linked hypohydrotic ectodermal dysplasia and recurrent infections, and identified hypomorphic mutations in NEMO. Two obligate carriers (mothers) in these families had clinical manifestations of incontinentia pigmenti. Doffinger *et al.* [13] identified osteopetrosis, lymphedema and multiple infections in the sons of women with incontinentia pigmenti. They then expanded their search to include children with extodermal dysplasia and infections, especially those caused by mycobacteria [13]. Jain *et al.* [14] were looking for unexplained cases of hyper-immunoglobulin M (IgM) syndrome and identified a small subgroup with ectodermal dysplasia and immunodeficiency (ED-ID). All these investigators identified

Figure 2. Teeth of a patient with a mutation in NFκB essential modulator (NEMO), demonstrating hypodontia and conical incisors.



Picture compliments of Dr Steven M. Holland (Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA).

hypomorphic mutations in NEMO that appeared to result in the immune deficiency and the other phenotypic findings.

X-linked anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) [13] or hyper-IgM syndrome with ectodermal dysplasia [15] is a rare congenital disease, caused by mutations in NEMO. The disease is characterized by abnormal development of ectoderm-derived skin appendages and susceptibility to infections. According to Orange *et al.* [16] the estimated incidence of ED-ID as a result of NEMO mutations is 1 in 250 000 live male births. Affected patients present with hypotrichosis or atrichosis, hypohidrosis or anhidrosis, leading to heat intolerance, and hypodontia or anodontia with conical incisors (Fig. 2) [13,15,16,17•,18]. In one of the initial reports of XL-EDA-ID a subset of patients presented with osteopetrosis and lymphedema [13], which was attributed to the disrupted signal transduction of the ectodysplasin A/ectodysplasin A receptor pair, which is critical for ectodermal development.

XL-EDA-ID patients are susceptible to infections with pyogenic bacteria, non-tuberculous mycobacteria and histoplasma. Routine immunologic evaluation shows an impaired antibody response to polysaccharide antigens. A subset of patients may have low IgA and IgG levels and normal to high IgM levels compatible with the immunoglobulin profile of hyper-IgM syndrome [5••, 13,15,16,18,19]. The ED phenotype results from impaired NFκB activation by the single ectodysplasin receptor. In contrast, immunodeficiency results from impaired NFκB activation in response to Toll-like receptor (TLR), interleukin-1 receptor and tumor necrosis factor (TNF) α receptor signaling.

Thus, the etiology of immunodeficiency is due to a combination of disrupted innate immunity and defective acquired immunity as a result of the host of receptor–ligand pairs that signal though the NFκB [20•].

Natural history of immunodeficiency due to mutations in nuclear factor κB essential modulator

Immunodeficiency with both cellular and humoral immune components has been described in affected boys. Given the fact that NFκB is required for signaling of many cytokines, as well as CD40L and TLRs, a link was established between this phenotype, susceptibility to infection and impaired NFκB activation. The majority of affected boys had mutations affecting the tenth exon, which encodes a zinc-finger domain, whereas a small subset of patients had point mutations outside this domain.

Susceptibility to infections by pyogenic bacteria early during infancy is typical. Common pathogens include *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella*, *Salmonella* and *Pseudomonas* species. These infections are usually in the form of bacterial sepsis, pneumonia, otitis or sinusitis. Lymphadenitis and bronchiectasis due to repeated pyogenic infections of lungs are among the reported complications in this disorder [5••,13,15, 16,17•].

Disseminated mycobacterial disease due to increased susceptibility to atypical mycobacteria has been described and *Mycobacterium avium* intracellulare, *Mycobacterium abscessus* and *Mycobacterium bovis* have been reported in these patients. TNFα, interferon-γ and interleukin-12 represent cytokines critical in the immune response to mycobacteria and all are regulated via NFκB. This serves as an explanation for the vulnerability of XL-EDA-ID patients to infections with atypical mycobacteria [16,18,21,22].

Viral infections reported in NEMO mutants include cytomegalovirus (systemic infection and colitis), Herpes simplex virus (stomatitis, pharyngitis and encephalitis), adenovirus (gastroenteritis), *Molluscum contagiosum* virus and Human papilloma virus. Herpes simplex virus-1 encephalitis has been reported to be fatal despite prompt treatment with high-dose acyclovir. This most likely indicates an increased susceptibility to Herpes simplex virus infections as part of the immunodeficiency. *Pneumocystis carinii* has also been described as an opportunistic infection in affected patients [16].

In the initial series published, immunologic assessments showed hypogammaglobulinemia with variable IgM and IgA levels together with impaired specific antibody production [12,13,15]. Coombs-positive hemolytic anemia

caused by the formation of IgM cold agglutinins in a patient with NEMO with mutation and immunodeficiency but without ectodermal dysplasia was reported by Niehues *et al.* [23]. Another case of acute hemolysis that required splenectomy was described in a patient. Large-joint arthritis and inflammatory bowel disease-like colitis were among the reported autoimmune problems. This is not surprising since autoimmune phenomena are not uncommon in immunodeficient patients, including those with hyper-IgM syndrome associated with defective CD40-mediated signaling.

In-vitro studies demonstrate impairments in CD40-mediated B-cell activation and isotype-class switching [5**,16,18]. B-cells show a complete lack of memory B-cells with an immature $IgD^{+}CD27^{-}$ phenotype [15]. Lymphocyte subsets are usually normal with only occasional T-cell lymphopenia. Lymphocyte-proliferative responses are almost always impaired. Natural killer cell cytotoxicity is usually poor, which may be an additional reason for increased susceptibility to viral infections in this immunodeficiency [24].

In addition to impaired ectodysplasin A receptor signaling, cells from XL-EDA-ID patients with NEMO mutations fail to demonstrate nuclear translocation of NFκB upon stimulation with TLR-specific ligands. The finding of diminished or absent in-vitro production of TNF and interleukin-12 in response to stimulation with the TLR-4 ligand lipopolysaccharide, helps to identify that there is also a defect in innate immunity associated with this syndrome.

Immunopathology in mutants of nuclear factor κB essential modulator

Patients with NEMO mutations are unable to produce normal interleukin-12 and TNFα in response to CD40L signaling; their B-cells are often incapable of class-switch recombination, which can lead to a humoral phenotype resembling the hyper-IgM with normal to high IgM and low IgA and IgG levels as well as an impaired antibody response to polysaccharide antigens [18]. A lack of somatic hypermutations and a lack of class-switch recombination in patients with mutations in the zinc finger domain have been explained by the critical regulatory role of this domain for CD40-mediated activation of NFκB [14].

The most common NEMO mutation that is described as leading to XL-EDA-ID, C417R, completely blocks specific antibody production whereas other mutations seem to allow for some specific antibody production [13,15,16,24]. This may be attributed to differential binding of upstream regulatory proteins to distinct regions of the protein. This also explains how germ-line mosaicism or distinct mutations affecting the leucine zipper region of this protein (while leaving the zinc-finger region intact) can cause immunodeficiency without impairing ectodermal development [17*]. In this case, signal transduction essential for normal immune development would be impaired whereas signals critical for ectodermal development remain intact. Mutations affecting the splice sites may lead to a milder clinical phenotype. These should be reviewed along with functional data since leakage through these mutations may lead to milder clinical phenotypes. To clearly understand the implications of a given mutation, in-vitro functional data should include evaluation of different TLRs.

Nishikomori *et al.* [25] reported an XL-EDA-ID patient with atypical features of very few naïve T-cells and defective proliferation of peripheral blood mononuclear cells and two populations of T, B and natural killer cells detected with normal and reduced expression of NEMO. This was described as the reversion mosaicism of a large gene duplication [25]. The somatic mosaicism was not due to fetomaternal transfusion but was most likely due to postzygotic reversion.

Osteopetrosis and lymphedema in patients with mutations in nuclear factor κB essential modulator

Receptor activator of NFκB (RANK) is normally expressed in osteoclast precursors and is involved in their differentiation and function. $RANK^{-/-}$ mice show an arrest in osteoclast differentiation and suffer from severe osteopetrosis. Based on these data, Doffinger *et al.* [13] hypothesized that their patients with lymphedema and osteopetrosis might have impaired RANK signaling [13]. Vascular endothelial growth factor-3 also signals through NFκB, impaired signaling of this protein may lead to lymphedema.

Incontinentia pigmenti due to mutations in nuclear factor κB essential modulator

Familial incontinentia pigmenti, a genodermatosis that segregates as an X-linked dominant disorder, causes in-utero male lethality [26,27**]. Incontinentia pigmenti has an incidence of between 1 in 10 000 and 1 in 100 000 and it is highly variable in presentation. In affected females it affects organs and tissues of ectodermal and mesodermal origin and leads to malformations of the skin, hair, nails, skeleton, teeth, eyes and central nervous system. The findings in skin include perinatal inflammatory vesicles, verrucous patches, swirled patterns of hyperpigmentation and dermal scarring. Problems with tooth eruption and alopecia are common whereas retinal dysplasia and neurological signs occur in a small percentage of cases.

Smahi *et al.* [28] and Zonana *et al.* [12] demonstrated that mutations in NEMO/IKKγ are responsible for most cases

Table 1. Features of disease associated with nuclear factor κB essential modulator.

Disease	Gene	Protein	Inheritance	Features of immunodeficiency ED	Common features or location of the mutation
ED-ID	IKKγ	NEMO	X-linked recessive	Combined immunodeficiency, profound and variable; variable features of ED*	Hypomorphic mutation located to coiled coils 1, 2, †zinc finger or IKK-binding domains
ED-ID	IKKγ	NEMO	X-linked recessive	Combined immunodeficiency, diverse and profound; variable features of ED with osteopetrosis and lymphedema	Hypomorphic mutation of the stop codon (X420W)
Immunodeficiency	IKKγ	NEMO	X-linked recessive	Combined immunodeficiency, usually profound and variable; no ED	Splice-site mutation affecting the leucine zipper domain
Incontinentia pigmenti	IKKγ	NEMO	X-linked dominant	No immunodeficiency; ED: dermal scarring, hyperpigmentation; lethal for males *in utero*; females have extremely skewed X inactivation in peripheral blood	Amorphic mutation; 70–80% genomic rearrangement leading to a truncated protein
ED-ID	NFKBIA	IκBα	Autosomal dominant	Combined immunodeficiency with ED	Gain of function, IκB cannot be removed from nuclear factor κB

ED, ectodermal dysplasia; ED-ID, ectodermal dysplasia with immunodeficiency; IκBα, inhibitor of nuclear factor κB; IKK, inhibitor of nuclear factor κB kinase; NEMO, nuclear factor κB essential modulator.
*Germline mosaicism reported to lead to a partial ED phenotype.
†Most common mutation is C417R, which completely blocks specific antibody production.

of familial incontinentia pigmenti and a new genomic rearrangement accounts for 70–80% of mutations leading to defective NFκB activation in incontinentia pigmenti cells. This rearrangement involves excision of the region between two repeated sequences located upstream of exon 4 and downstream of exon 10, resulting in the synthesis of a truncated inactive NEMO molecule (with exons 4–10 eliminated) [28,29]. This mutation arises predominantly by intrachromosomal misalignment during meiosis [30]. In incontinentia pigmenti patients with this rearrangement, fetally derived primary fibroblasts are unresponsive to NFκB-activating stimuli. IκB molecules do not degrade upon stimulation and they are very sensitive to TNFα-induced apoptosis.

This disorder is X-linked dominant and primarily affects females. However, there have been several cases of severe incontinentia pigmenti in males. Heterozygous females with incontinentia pigmenti have extremely skewed X inactivation and have surviving T-cells and B-cells expressing only the wild-type NEMO allele [31]. On the other hand, the X-inactivation skewing is less complete in the skin. Three mechanisms have been proposed to explain the survival of affected male patients: the half-chromatid hypothesis, unstable premutation and a higher rate of de-novo germline mutations. However, the reasons for cell death in females and in-utero lethality in males are not truly known.

Mutations in inhibitory κBα are distinct in terms of immunodeficiency

More recently, Courtois *et al.* [32] reported a male patient carrying a dominant gain-of-function mutation in IκBα producing a profound T-cell deficiency characterized by lack of CD45RO+ cells and impaired in-vitro lymphocyte stimulation via T-cell receptor [32]. Like boys with a NEMO mutation, he was unable to respond to TLR and TNF receptor stimulation, had impaired antibody production. The point mutation, S32I substitution, was in one allele of IκBα and resulted in the inability of the mutant IκBα to be phosphorylated. This serine residue is critical for IKK phosphorylation and for phospho-IκBα ubiquitination and degradation [33]. This patient had susceptibility to both Gram-positive and Gram-negative bacteria. However, unlike patients with the NEMO mutation, he had normal natural killer cell activity and did not have mycobacterial infection. The patient was transplanted at age 1 year, and clinical manifestations of ectodermal dysplasia appeared 2 years later. Although NEMO and IκBα-deficient patients share some clinical manifestations of ectodermal dysplasia, their immunological phenotypes are distinct [20•] (Table 1).

Conclusion

Until recently we could only speculate about the genetic diseases caused by NFκB dysfunction. This was changed with the identification of the X-linked gene NEMO/IKKγ, encoding a regulatory molecule of the NFκB signaling pathway. We now know that NEMO/IKKγ mutations lead to complex immunopathology. Amorphic mutations in this gene cause incontinentia pigmenti in females whereas hypomorphic mutations lead to ED-ID. Genetic discoveries and clinical studies have helped clarify the role of NFκB in skin innate and acquired immunity as well as skin homeostasis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 572–573).

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