Allergology International. 2012;61:207-217
DOI: 10.2332/allergolint.12-RAI-0446

REVIEW ARTICLE

Review Series: Primary Immunodeficiency and Related Diseases

Diagnosis and Treatment in Anhidrotic Ectodermal Dysplasia with Immunodeficiency

Tomoki Kawai[1], Ryuta Nishikomori[1] and Toshio Heike[1]

ABSTRACT

Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) is characterized according to its various manifestations, which include ectodermal dysplasia, vascular anomalies, osteopetrosis, and diverse immunological abnormalities such as susceptibility to pathogens, impaired antibody responses to polysaccharides, hypogammaglobulinemia, hyper-IgM syndrome, impaired natural killer cell cytotoxicity, and autoimmune diseases. Two genes responsible for EDA-ID have been identified: *nuclear factor-κB (NF-κB) essential modulator (NEMO)* for X-linked EDA-ID (XL-EDA-ID) and *IκBα* for autosomal-dominant EDA-ID (AD-EDA-ID). Both genes are involved in NF-κB activation, such that mutations or related defects cause impaired NF-κB signaling. In particular, NEMO mutations are scattered across the entire NEMO gene in XL-EDA-ID patients, which explains the broad spectrum of clinical manifestations and the difficulties associated with making a diagnosis. In this review, we focus on the pathophysiology of EDA-ID and different diagnostic strategies, which will be beneficial for early diagnosis and appropriate treatment.

KEY WORDS

anhidrotic ectodermal dysplasia with immunodeficiency, immunodeficiency, inflammation, NEMO, NF-kappaB inhibitor alpha

INTRODUCTION

Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) is a primary immunodeficiency disorder in which patients present with various manifestations, such as EDA, vascular anomalies, and osteopetrosis.[1-5] The immunological features of EDA-ID include susceptibility to pathogens, impaired antibody response to polysaccharrides, hypogammaglobulinemia, hyper IgM syndrome, impaired natural killer (NK) cell cytotoxicity, and autoimmune diseases.[6] Two genes responsible for EDA-ID have been identified: nuclear factor-κB (NF-κB) essential modulator (NEMO) in X-linked EDA-ID (XL-EDA-ID) and IκBα in autosomal-dominant EDA-ID (AD-EDA-ID). Both genes are involved in NF-κB activation such that mutations or related defects cause impaired NF-κB signalling.[5,7] For the appropriate diagnosis and treatment of EDA-ID, the physicians should be well aware of the broad spectrum of its clinical phenotypes. Moreover, in the genetic diagnosis of XL-EDA-ID, the potential presence of a NEMO pseudogene and the occurrence of somatic mosaicism must be considered. In this review, we focus on the variable clinical manifestations of XL-EDA-ID and the diagnostic precautions that can be taken in individuals at risk for the disease.

ETIOLOGY OF EDA-ID

The first case of EDA-ID, in a boy who died of miliary tuberculosis, was reported by Frix *et al*. in 1986.[8] The second case involved a boy who suffered from multiple life-threatening infections caused by *Pseudomonas aeruginosa*, *Mycobacterium avium*, and cytomegalovirus infections.[3] In spite of extensive searches for the cause of the refractory infections in these patients,

[1]Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan.
Conflict of interest: No potential conflict of interest was disclosed.
Correspondence: Ryuta Nishikomori, MD, PhD, Department of Pediatrics, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606–8507, Japan.
Email: rnishiko@kuhp.kyoto-u.ac.jp
Received 21 March 2012.

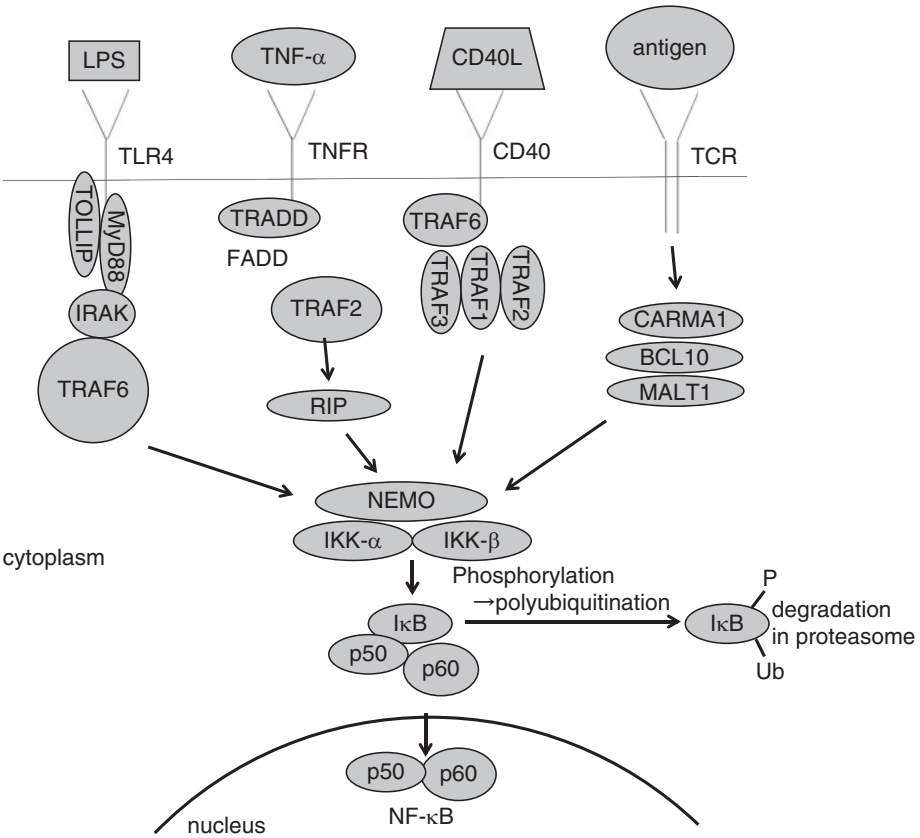

Fig. 1 NF-κB activation pathways associated with NEMO and IκBα. The major molecules involved in the TLR-4, TNFR, CD40, and TCR signalling pathways and NEMO-mediated NF-κB activation are depicted. TOLLIP, Toll-interacting protein; MyD, myeloid differentiation factor; IRAK, interleukin-1 receptor-associated kinase; TRAF, tumor necrosis factor receptor-associated factor; TRADD, tumor necrosis factor receptor-associated death domain; FADD, fas-associated protein with death domain; RIP, receptor-interacting protein; CARMA, caspase recruitment domain-containing membrane-associated guanylate kinase protein; BCL, B-cell lymphoma protein; MALT, mucosa-associated lymphoid tissue lymphoma translocation protein; Ub, poly-ubiquitin chain; P, phosphate.

immunological dysfunctions were not identified. In 1996, Abinun *et al*. described a young male patient with EDA-ID who had an impaired antibody response to polysaccharide antigens.[2] Their report was the first to shed light on the mechanism of EDA-ID-associated immunodeficiency. In 2001, three groups were able to show that defects in the NEMO gene are responsible for XL-EDA-ID. Those authors demonstrated that the clinical manifestations of XL-EDA-ID, including EDA and the immunological dysfunctions, were caused by impaired NF-κB activation due to the identified genetic alterations.[5,9,10] In addition, in a 2003 paper by Courtois *et al*., the etiology of AD-EDA-ID was determined to be a heterozygous gain-of-function mutation in the IκBα gene.[7] As both forms of EDA-ID are typically diagnosed by genetic testing, NEMO and IκBα mutations have been linked to a broad spectrum of clinical phenotypes.[11] Currently, the estimated incidence of XL-EDA-ID is 1 : 250,000 live male births.[6] In AD-EDA-ID, six patients and four IκBα mutations have been reported thus far.[7,12-15]

THE ROLES OF NEMO AND IκBα IN THE NF-κB ACTIVATION PATHWAY

NF-κB transcription factors are critical regulators of immunity, the stress response, apoptosis, and differentiation. Mammalian cells make use of two main NF-κB activation pathways, the canonical pathway and the non-canonical pathway. The canonical pathway, in which NEMO and inhibitors of NF-κB (IκB) are essential control elements, is induced by most physiological NF-κB stimuli.[16] NEMO and IκB are also involved in the non-canonical NF-κB activation pathway, albeit indirectly.[17] Homo- or heterodimers of NF-κB proteins (p50, p52, RelB, and c-rel) are normally retained in the cytoplasm through interactions

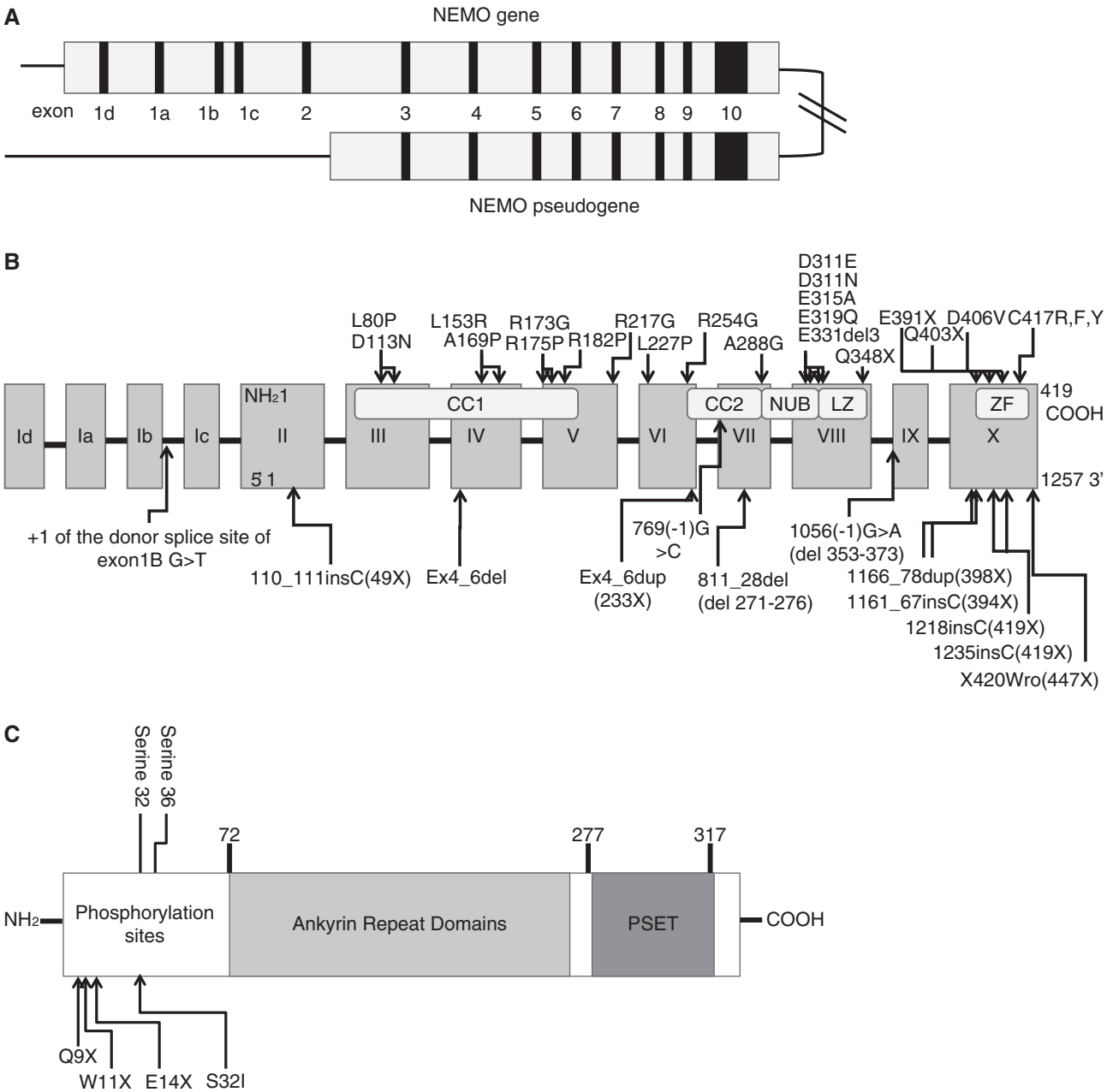

Fig. 2 Schematic representations of the normal NEMO and IκBα genes. (**A**) The normal NEMO gene and a NEMO pseudogene. Schematic representation of the coding-region domain and reported mutations in NEMO (**B**) and IκBα (**C**).

with IκB family proteins, which consist of IκBα, IκBβ, and IκBε. In response to the appropriate signals, these three proteins are phosphorylated, polyubiquitinated, and degraded though the ubiquitin-proteasome pathway (Fig. 1), thereby freeing NF-κB to translocate to the nucleus where it activates its target genes.[16]

The phosphorylation event in this sequence is carried out by a high molecular mass, multiprotein kinase complex containing two subunits with kinase activity (IKK1/α and IKK2/β) and NEMO (IKK3/γ). The human NEMO gene, located at Xq28, is a 23-kb gene structured in nine exons and four alternative non-coding first exons. A non-functional copy of the NEMO gene, IKBKGP (also referred to as the NEMO pseudogene), is located 31.6 kb distal to exon 10 (Fig. 2A). IKBKGP maps within a 35.7-kb duplicated fragment that is oriented tail to tail with the NEMO gene and contains exons 3-10, with 99.8% homology.[18] The ~48-kDa NEMO protein is composed of two coiled coil (CC1, CC2) domains, a leucine-zipper (LZ) domain, a NEMO ubiquitin-binding

Table 1 Clinical and immune function associated with hypomorphic NEMO mutations in reported cases and Japanese cases

Functional or clinical category	Modified - Hanson *et al.*[11]	Japanese cases
Ectodermal dysplasia	40/52 (77%)	9/10 (90%)
Osteopetrosis	5/65 (8%)	0/10 (0%)
Lymphedema	5/65 (8%)	1/10 (10%)
Autoimmune/inflammatory disease	14/66 (23%)	5/10 (50%)
Dead	24/66 (36%)	2/10 (20%)
Infectious susceptibility	60/61 (98%)	10/10 (100%)
Bacterial infection	45/52 (86%)	10/10 (100%)
Mycobacterial infection	23/52 (44%)	4/10 (40%)
Pneumocystis pneumonia	4/52 (21%)	0/10 (0%)
DNA-virus infection	11/52 (21%)	1/10 (10%)
Meningitis	12/61 (21%)	1/10 (10%)
Pneumonia	19/61 (31%)	3/10 (30%)
Sepsis/bacteremia	20/61 (33%)	5/10 (50%)
Abscess	18/61 (30%)	3/10 (30%)
Hypogammaglobulinemia	24/41 (59%)	N.D.
Specific antibody deficiency	18/28 (64%)	N.D.
Impaired antibody response to polysaccharide	13/16 (94%)	3/3 (100%)
Impaired NK cell cytotoxicity	10/10 (100%)	N.D.

(NUB) domain, and a zinc finger (ZF) domain (Fig. 2B).[19] NEMO has no apparent catalytic activity but is instead required in activation of the kinase complex in response to extracellular (or intracellular) stimuli, such as members of the TIR (TLR-ligands, IL-1β, and IL-18), and TNFR (TNF-α, LTα1/β2, and CD154) superfamilies.[5] The protein interacts with the IKK complex through the N-terminal portion of its CC1. Upon cytokine signalling, Lys-63-linked or linear ubiquitin chains bind the NUB and ZF domains; the latter bears a second ubiquitin-binding site. This interaction with ubiquitin promotes the recruitment and oligomerisation of NEMO, with the latter achieved by the assembly of the CC2/LZ portion of NEMO. After inducing upstream signalling, CC2/LZ converts to its fully folded conformation and forms oligomers of NEMO, which activate the IKK complex and lead to the phosphorylation of IκB family proteins.[19-22]

Hypomorphic mutations of NEMO impair IκBα phosphorylation and the sequential activation of NF-κB, resulting in the variable clinical features of EDA-ID. By contrast, amorphic mutations of NEMO are lethal in males and result in incontinentia pigmenti in females.[23,24] The multiple functional domains of NEMO may explain why NEMO mutations are scattered throughout the NEMO gene as well as the broad spectrum of clinical phenotypes.[11]

The IκBα protein, a member of the serine/threonine protein kinase family, contains phosphorylation sites at its N-terminal, ankyrin repeat domains in its central portion, and, at its C-terminal, repeated peptidic sequence rich in proline, glutamic acids, serine, and threonine (rPEST) domains (Fig. 2C).[7] IκBα inhibits activation of the NF-κB complex while phosphorylation of Ser32 and Ser36 in its phosphorylation domains triggers IκBα ubiquitination, leading to degradation of the protein within the proteasome and, in turn, the nuclear translocation of NF-κB and subsequent activation of its target genes.

Hypermorphic mutations of IκBα impair its phosphorylation such that mutant IκBα molecules accumulate in the cytoplasm, thereby inhibiting the nuclear translocation of NF-κB and target-gene activations.[7] All of the reported IκBα mutations were shown to cause abnormalities in the phosphorylation site of IκBα, resulting in the abnormal accumulation of the protein and therefore the retention of NF-κB in the cytoplasm.

CLINICAL MANIFESTATIONS OF XL-EDA-ID

NF-κB is involved in many forms of signal transduction, including pathways involving interleukin 1 (IL-1) family protein receptors, Toll-like receptor, vascular endothelial growth factor receptor-3 (VEGFR-3), receptor activator of nuclear factor κB (RANK), the ectodysplasin-A receptor, CD40, and the tumour necrosis factor (TNF) receptor.[16] Consequently, mutations in NEMO cause abnormalities of these routes of signal transduction, and thus the clinical features documented in XL-EDA-ID patients. The clinical manifestations of XL-EDA-ID described by Hanson *et al*. and those of Japanese cases are shown in Table 1.

EDA

The development of cell types and tissues of ectodermal origin, such as keratinocytes, hair follicles, and sweat glands, is associated with the ectodysplasin/ectodysplasin receptor signalling pathway. Ectodyspla-

sin, a member of the TNF family, is encoded by the *ED1* (formerly the *EDA*) gene. The ectodysplasin receptor is homologous to members of the TNF receptor superfamily and is encoded by the *DL* [the ortholog of the mouse downless gene (*dl*)] gene. Mutations in *ED1* are responsible for the X-linked recessive type of EDA, and mutations in *DL* for the autosomal recessive and autosomal-dominant types of the disease. NF-κB activation is an essential step in the ectodysplasin/ectodysplasin signalling pathway. Mutations in NEMO or IκBα impair this pathway, resulting in the various manifestations of EDA in affected patients.[5]

A clinical diagnosis of EDA is obtained when at least two of the following seven characteristics are observed: (1) decreased skin pigment, (2) periorbital wrinkling and hyperpigmentation, (3) sparse to absent hair, (4) hypoplastic to absent sweat glands, (5) hypodontia to anodontia with a tendency to delayed eruption, resulting in a deficient alveolar ridge or conically shaped anterior teeth, (6) low nasal bridge, small nose with hypoplastic alae nasi, and (7) full forehead with prominent supraorbital ridges.[11]

Interestingly, although EDA is one of the characteristic signs of EDA-ID, it is not always apparent during early infancy and is totally absent in some patients (Table 1). In these cases, recognition of the typical immunological abnormalities should be followed by genetic analysis of the NEMO and IkBα genes.

OSTEOPETROSIS AND VASCULAR ANOMALIES

Osteopetrosis and vascular anomalies are observed in patients with severe phenotypes of XL-EDA-ID. This form of the disease is called EDA-ID with osteopetrosis and lymphedema (OL-EDA-ID). Most of these patients present with failure to thrive and refractory infections, including *Pneumocystis* pneumonia, necessitating hematopoietic stem cell transplantation (HSCT) to avoid premature death from related complications.[5,11,25]

In various animal models, RANKL- and TNF-induced NF-κB signalling were shown to influence osteoclastogenesis in the bone marrow. In humans with XL-EDA-ID, the characteristic osteopetrosis can be explained by the inhibition of osteoclastogenesis due to impaired RALKL-induced signalling and susceptibility to TNF-α-induced apoptosis of osteoclast precursors, as a consequence of NEMO mutations.[5,26]

Mutations in VEGFR-3 were shown to cause primary lymphedema due to the related vascular anomalies and the fact that VEGFR-3 signalling induces NF-κB activation. The lymphedema observed in OL-EDA-ID may reflect severe dysfunctional NF-κB activation, likewise caused by NEMO mutations.[5]

SUSCEPTIBILITY TO BACTERIAL AND VIRAL INFECTIONS

Most XL-EDA-ID patients present with increased susceptibility to infections, particularly those of bacterial origin. Although hypogammaglobulinemia occurs in only 59% of the patients, in most of them the impairment consists of the failure to mount a specific antibody response to pneumococcal polysaccharides, resulting in susceptibility to pyogenic bacteria including *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Staphylococcus aureus*.[11]

Also in EDA-ID, the observed deficiencies in innate immunity, i.e., the increased susceptibility to bacterial and viral infections, are caused by the impaired cellular responses to various stimuli, including TNF-α, IL-1β, IL-18, and lipopolysaccharides.[5] Moreover, CD40-mediated signals are partially impaired in both dendritic cells and B cells, which likewise leads to an impaired antibody response.

SUSCEPTIBILITY TO MYCOBACTERIA

Some XL-EDA-ID patients are particularly vulnerable to mycobacterial infections, which are one of the most serious complications associated with the disease. Infections with the various mycobacterial species, among which *Mycobacterium avium intracellulare* is the most commonly reported,[11] manifest as cellulitis, osteomyelitis, lymphadenitis, pneumonia, and disseminated diseases. In Japanese cases of XL-EDA-ID, two of four patients with mycobacteria infection were positive for bacillus Calmette-Guerin (BCG). Therefore, the treating physician should make sure that he or she is appropriately vaccinated.

The increased frequency of mycobacterial infections in XL-EDA-ID patients can be ascribed to an intrinsic defect of T cell-dependent IL-12 production by monocytes, resulting in defective IFN-γ secretion by T cells. IL-12 production is also impaired as the result of a defect in NEMO-mediated CD40 signalling by monocytes and dendritic cells.[5,27,28]

DEFECTIVE NK CELL CYTOTOXICITY

XL-EDA-ID patients have impaired NK cell cytotoxicity although the number of NK cells in the peripheral blood is normal. In fact, the identification of an NK cell defect may be considered as diagnostic of XL-EDA-ID in the presence of the corresponding clinical features.[11,29] This abnormality was partially reversed by the *in vitro* addition of IL-2. Signalling by NKp30 is associated with NF-κB activation in the canonical pathway. NKp30 is one of the natural cytotoxicity receptors, which are major receptors expressed almost exclusively on human NK cells. The defects in NK cell cytotoxicity in patients with NEMO mutations can be explained by the impaired NF-κB activity in the canonical pathway, which is induced after the ligation of specific activating receptors, including NKp30.[30] Interestingly, defective NK cell cytotoxicity

has not been found in AL-EDA-ID patients.[7]

Finally, defective NK cell cytotoxicity may also explain the increased susceptibility of XL-EDA-ID patients to infections with the herpes group of viruses.

INFLAMMATORY DISEASES

Inflammatory disorders and autoimmunity are often observed in XL-EDA-ID, with inflammatory colitis (called NEMO colitis) accounting for 25% of the cases in these patients.[11] NEMO colitis, which usually occurs early in childhood, causes intractable diarrhoea and failure to thrive. Histological examination shows active colitis with abundant neutrophil infiltration.[31-33]

In searching for the mechanisms underlying the association of NEMO colitis with XL-EDA-ID, Nanci *et al*. produced a mouse model based on a conditional NEMO knockout in the gut epithelium. NEMO-deficient epithelial cells were shown to be sensitive to TNF-α-induced apoptosis and accounted for the severe chronic intestinal inflammation. Accordingly, the authors suggested that the impaired NF-κB signalling in EDA-ID resulted in TNF-α induced apoptosis and subsequent inflammatory diseases.[34]

PROGNOSIS

According to the database of XL-EDA-ID, the mean age at death is 6.4 years. In more recent cases, death has occurred even earlier, although this is probably an artifactual finding reflecting earlier recognition of the disease based on its improved diagnosis.[11]

DIAGNOSIS OF XL-EDA-ID

In the many XL-EDA-ID patients with normal immunological findings, early diagnosis of the disorder is particularly difficult.[1-4,8] However, since EDA is characteristic and diagnostically useful in EDA-ID patients,[4] recognition by the physician of its signs in an infant warrants a genetic analysis. Nevertheless, EDA is not a consistent finding and even if the characteristic signs are absent, EDA-ID should not be excluded.[35] For example, if the patient suffers from recurrent bacterial infections or environmental mycobacterial infections, XL-EDA-ID should be included in the differential diagnosis. In this setting, the analysis of NK cell cytotoxicity could be helpful.[29]

NEMO mutations are scattered throughout the NEMO gene (Fig. 1C), which accounts for the numerous clinical phenotypes of EDA-ID. Indeed, genotype-phenotype correlations have been shown in recent studies. Thus, genotyping might, at least to some extent, serve to predict the EDA-ID phenotype in affected patients.

The presence of the NEMO pseudogene makes it difficult to perform genetic analysis using genomic DNA with Sanger sequencing. Instead, NEMO mutations should be identified by sequencing analysis of NEMO cDNA. Large deletion or duplication mutations in the NEMO gene have been detected in some cases of XL-EDA-ID[36] and in the majority of patients with incontinentia pigmenti.[23] Therefore, additional molecular methods, including Southern blotting analysis, and detailed PCR analyses can provide important diagnostic information.

As noted above, the coding sequence of NEMO cDNA extends from exon 2 to exon 10. Lymphocytes express NEMO transcripts comprising exons 1A, 1B, or 1C spliced to exon 2, with exon1B transcripts making up the majority of the three isoforms. In a case report of a NEMO deficiency, a mutation at position +1 of the donor splice site of exon 1B resulted in aberrant NEMO mRNA and the reduced expression of a normal NEMO protein.[37] Therefore, genomic sequencing of all ten NEMO exons, i.e., including exon 1, is necessary in the genetic diagnosis of XL-EDA-ID.

Despite ample genetic knowledge of the defects in XL-EDA-ID, the presence of somatic mosaicism in these patients poses a diagnostic challenge. Although only three cases of XL-EDA-ID involving somatic mosaicism have been published in the literature,[33,36,38] our recent study determined a much higher frequency.[39] Among the patients analysed by our group, somatic mosaicism was observed predominantly in T cells, which suggested that NEMO is critical to T cell proliferation. While the clinical impacts of somatic mosaicism in XL-EDA-ID have not been demonstrated, the presence of this form of the disease calls for care in its genetic diagnosis. Flow cytometric analysis of the NEMO protein is diagnostically useful for some, but not all of the NEMO mutations occurring in somatic mosaicism.[36,38]

CLINICAL MANIFESTATIONS OF AD-EDA-ID

Mutations of IκBα cause signal transduction abnormalities that are associated with NF-κB activation, resulting in various clinical manifestations, analogous to mutations in NEMO.[7]

In AD-EDA-ID, four mutations in the IκBα gene have been reported, p.Ser32Ile[7,12] and three nonsense mutations p. Gln 9 X, p. Trp 11 X, and p.Glu14X.[13-15] Among the AD-EDA-ID patients reported in the literature, EDA was a consistent finding, except in patients with IκBα p.Ser32Ile mosaicism (Table 2). This latter group suffered from severe recurrent bacterial infections, *Pneumocystis jiroveci* infection, and cutaneous candidiasis. Hypogammaglobulinemia with no specific antibodies, reduced TCRγδ T cells, and low T cell proliferation in response to anti-CD3 were determined as well. Furthermore, although a deficiency in NK cell cytotoxicity is seen in most NEMO-deficient patients, it was not detected in patients with the p.Ser32Ile mutation.[7,12]

A pediatric patient with somatic mosaicism involving the p.Ser32Ile IκBα mutation presented with juvenile idiopathic arthritis and was subsequently treated with steroid administration for 10 years during childhood. As an adult, he presented with tentative

Table 2 Clinical symptoms and immune functions associated with the various IκBα

Mutations of IκBα	p.32Ile	p.32Ile mosaicism	Gln9X	Trp11X	Glu14X
EDA	+	-	+	+	+
Failure to thrive	+	-	+	-	+
Bacterial infection	Severe	Episodic *S. typhimurium* infection	Severe	Recurrent pneumonia	Severe
Pneumocystic jiroveci infection	+	-	-	-	+
Cutaneous candida infection	+	-	-	-	+
Autoimmune or inflammatory disease	-	Systemic JIA (in childhood) RF(+) oligoarthritis (in adulthood)	Inflammatory bowel disease	-	-
Treatment	IVIG HSCT	Steroid Non-steroidal-anti-inflammatory drugs	IVIG HSCT (scheduled)	Healthy with IVIG	IVIG HSCT (died due to sepsis)
Gammaglobulin abnormality	Hypogamma-globulinemia	-	Increased IgA	Increased IgA, decreased IgM	-
Specific antibody deficiency	+	-	n.d.	+	n.d.
Lymphocytosis	+	-	+	-	+
Abnormal lympho-cyte proliferation	Normal (PHA) Reduced (CD3, candi-din, tetanus)	Mildly reduced (CD3,PHA)	Reduced (PHA, Con-A)	Normal (PHA, CD3, CD3/ CD28, tetanus, diphtheria)	Normal (PHA,PWM, Con-A, tetanus)
TCRγδ	Decreased	Normal	n.d.	n.d.	Decreased
NK cell abnormali-ties	Normal NK cell activity	n.d.	n.d.	Normal percent-age of NK cells	Reduced NK cells
Impaired TLR response	+		+	+	+

rheumatoid-factor-positive oligoarthritis. An episodic *Salmonella typhimurium* infection was effectively treated with antibiotics and the patient has since been healthy.[12] This case suggests that somatic mosaicism in the p.Ser32Ile mutation accounts for the autoimmune disorders seen in some EDA-ID patients.

The patients with the three nonsense mutations (p.Trp11X, p.Gln9X, and p.Glu14X) had a normal IgG levels. The patient with the p.Glu14X mutation presented with failure to thrive since early childhood and suffered from recurrent bacteremia and *Pneumocystis jiroveci* infections. The p.Glu14X mutation causes a downstream re-initiation of translation of IκBα mRNA. The resulting N-terminally truncated protein lacks both serine phosphorylation sites (Ser32 and Ser36) and inhibits NF-κB activation by working as a dominant negative repressor in lymphocytes and monocytes.[14] The patient with the p.Gln9X mutation had suffered from recurrent viral and bacterial infections beginning in early childhood and later from inflammatory bowel disease.[15] The patient with p.Trp11X mutation presented with recurrent pneumonia and bronchiectasis but no history of bacteremia or mycobacterial infections. She had been healthy following the initiation of immunoglobulin infusion therapy, at the age of 10 years.[13] Similar to p.Glu14X, p.Trp11X and p.Gln9X manifest as downstream re-initiation mutations. However, why the three nonsense mutations give rise to three distinct clinical pictures remains to be explored.

DIAGNOSIS OF AD-EDA-ID

As noted above, EDA is a diagnostically helpful manifestation of AD-EDA-ID because it is seen in all of these patients, except in those with somatic mosaicism. Recurrent severe infections with various pathogens are common, including bacteria, virus, fungi, and, in young infants, *Pneumocystis jiroveci*.[7,12-15] Although the immune dysfunctions seen in AL-EDA-ID are more severe than those typical of XL-EDA-ID, they are not diagnostically conclusive and cannot be used to distinguish between XL-EDA-ID and AD-

Table 3 Summary of reported cases of EDA-ID in which the patient underwent HSCT

Case	Mutation	HLA match	Source	Conditioning	Outcome	References
1	NEMO c.1167_1168 insC	UD 2/6 matched Disparate at HLA-A by serology, disparate at both HLA-A and both HLA-DR by DNA typing	CB	Fludarabine 150 mg/m^2 Melphalan 140 mg/m^2 rATG 12.5 mg/kg	Alive	31
2 (First HSCT)	NEMO c.1167_1168 insC	Matched sibling	PSC	Fludarabine 6 mg/kg/day Busulfan target 4000 μM/min i.v. × 2 days rATG 8 mg/kg	Graft failure	32
2 (Second HSCT)		Same donor	PSC	Fludarabine 5 mg/kg/day Melphalan 3.5 mg/kg Alemtuzumab 30 mg/kg	Alive Rash, diarrhea	
3	NEMO c.1167_1168 insC	UD 7/10 matched Disparate at HLA-B and both HLA-C	CB	Busulfan target 900-1300 μM/min i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg eATG 90 mg/kg	Alive	42
4	NEMO c.1259A>C	UD matched	BM	Busulfan 20 mg/kg Cyclophosphamide 200 mg/kg rATG 5 mg/kg	Died at day +6 from veno-occlusive disease	25
5 (First HSCT)	NEMO c.768 + 5G>A	UD matched	BM	Busulfan 1 mg/kg i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg rATG 9 mg/kg	Graft failure	32
5 (Second HSCT)		Same donor	PSC	Fludarabine 160 mg/m^2	Died at day +314 due to para-influenza type III virus infection	
6	NEMO c.458T>G	Matched sibling	BM	Busulfan target 900-1300 μM/min i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg	Alive Continued colitis	40
7	NEMO c.931C>G	UD Matched	BM	Fludarabine 150 mg/m^2 Melphalan 140 mg/m^2 rATG 5 mg/kg	Alive	38
8	NEMO duplication of exon 4-5	UD 5/8 locus matched Disparate at HLA-B by serology Disparate at HLA-A, B, and C by DNA typing	CB	Fludarabine 150 mg/m^2 Melphalan 140 mg/m^2 rATG 12.5 mg/kg	T-cell graft failure Died at day +60 due to sepsis	39
9 (First HSCT)	IκBα STOP codon Glu14	UD 8/10 locus matched Disparate at HLA-B and C	CB	Fludarabine 5 mg/kg Cyclophosphamide 200 mg/kg rATG 9 mg/kg	Graft failure	32
9 (Second HSCT)		UD 7/8 locus matched Disparate at HLA-A	CB	Busulfan 1.1 mg/kg i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg Alemtuzumab 36 mg/kg	Graft failure Died from sepsis due to *Pseudomonas aeruginisa*	
10	IκBα missense mutation at Ser 32	Maternal haploidentical	BM	Busulfan 20 mg/kg Cyclophosphamide 200 mg/kg	Alive	43

Abbreviations: rATG, rabbit antithymocyte globulin; eATG, equine antithymocyte globulin; UD, unrelated donor; CB, cord blood; PSC, peripheral stem cell; BM, bone marrow.

EDA-ID nor do they obviate the need for a genomic diagnosis of NEMO and IκBα in males with suspected EDA-ID.

TREATMENT

For most XL-EDA-ID patients and for all those with AD-EDA-ID, treatment should consist of intravenous or subcutaneous immunoglobulin administration because of the impaired antibody response to polysaccharides and the susceptibility to pyogenic bacterial infection seen in the two conditions, despite the presence of normal levels of specific antibodies against other pathogens.[2] In EDA-ID patients with suspected infections, early empirical intravenous antibiotic administration is essential as the disease also results in an inability to increase plasma C-reactive protein (CRP) concentrations and to mount a fever as part of the initial inflammatory response, due to the impairment of Toll-like receptor signalling.

Candida albicans and *Pneumocystis jirovecii* infections are seen in some XL-EDA-ID patients and in nearly all AD-EDA-ID patients.[5,7,14,25] In such cases, the early and adequate administration of antibiotic prophylaxis, with cotrimoxazole and anti-fungal drugs, is strongly recommended.

Chronic atypical mycobacterial infections are also frequent in XL-EDA-ID and they are associated with a poor prognosis.[11] These infections progress insidiously and are almost inevitably disseminated at the time of disease diagnosis. In the three Japanese patients with XL-EDA-ID and atypical mycobacterial infections, only one sign or symptom, i.e., lymphadenopathy (BCG-positive), failure to thrive (*Mycobacterium szulgai* infection), and intractable diarrhoea (bacillus Calmette-Guerin) led, respectively, to the correct diagnosis.[36,38] However, by that time, the mycobacterial infections had already disseminated, thus highlighting the importance of their periodic surveillance in EDA-ID patients. It should be noted that although most AD-EDA-ID patients show a severe immunodeficiency, atypical mycobacterial infections have not been reported, perhaps due to the early mortality or because HSCT was performed in early childhood. NEMO colitis often has a complicated course in XL-EDA-ID such that the quality of life of these patients is reduced considerably. Corticosteroids, but not antimicrobial agents have been shown to be effective in this setting.[40,41] In a case report, inflammatory colitis in an XL-EDA-ID patient was successfully treated with anti-TNFα antibody administration.[33] Although this approach is likely to increase the risk of mycobacterium infection, it may still be a therapeutic option in patients with NEMO colitis.

Two patients with AD-EDA-ID and combined immunodeficiency and eight patients with XL-EDA-ID of severe clinical phenotype underwent HSCT (Table 3).[25,32,38-40,42,43] In five of the patients with XL-EDA-ID and in one with AD-EDA-ID, both the immunodeficiency and long-term survival improved, whereas in two patients with XL-EDA-ID, the disease remained unmodified. Three XL-EDA-ID patients and one with AD-EDA-ID died after HSCT, one from veno-occlusive disease, one from para-influenza virus type III, one from septic shock, and one other from bacterial sepsis caused by a resistant strain of *Pseudomonas aeruginosa*. Three XL-EDA-ID patients and one AD-EDA-ID patient experienced graft failure. These cases suggest that EDA-ID patients have intrinsic difficulties with successful engraftment[32] such that novel therapeutic approaches to this heterogeneous genetic disorder are needed.

CONCLUSIONS

Patients with EDA-ID present with various pathologies, including a high susceptibility to infections, the extent of which depends partially on the underlying genotype of the disease. In XL-EDA-ID patients, NEMO mutations scattered across the entire NEMO gene have been identified. These no doubt explain the broad spectrum of clinical manifestations that are typical for XL-EDA-ID. Accordingly, a genetic analysis is critical for its early diagnosis and appropriate treatment.

REFERENCES

1. Pinheiro M, Freire-Maia N. Ectodermal dysplasias: a clinical classification and a causal review. *Am J Med Genet* 1994;**53**:153-62.
2. Abinun M, Spickett G, Appleton AL, Flood T, Cant AJ. Anhidrotic ectodermal dysplasia associated with specific antibody deficiency. *Eur J Pediatr* 1996;**155**:146-7.
3. Sitton JE, Reimund EL. Extramedullary hematopoiesis of the cranial dura and anhidrotic ectodermal dysplasia. *Neuropediatrics* 1992;**23**:108-10.
4. Abinun M. Ectodermal dysplasia and immunodeficiency. *Arch Dis Child* 1995;**73**:185.
5. Döffinger R, Smahi A, Bessia C *et al*. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet* 2001;**27**:277-85.
6. Orange JS, Jain A, Ballas ZK, Schneider LC, Geha RS, Bonilla FA. The presentation and natural history of immunodeficiency caused by nuclear factor kappaB essential modulator mutation. *J Allergy Clin Immunol* 2004;**113**:725-33.
7. Courtois G, Smahi A, Reichenbach J *et al*. A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest* 2003;**112**:1108-15.
8. Frix CD 3rd, Bronson DM. Acute miliary tuberculosis in a child with anhidrotic ectodermal dysplasia. *Pediatr Dermatol* 1986;**3**:464-7.
9. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. *Nat Immunol* 2001;**2**:223-8.
10. Mansour S, Woffendin H, Mitton S *et al*. Incontinentia pigmenti in a surviving male is accompanied by hypohidrotic ectodermal dysplasia and recurrent infection. *Am J Med*

Genet 2001;**99**:172-7.
11. Hanson EP, Monaco-Shawver L, Solt LA *et al*. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. *J Allergy Clin Immunol* 2008;**122**:1169-77. e16.
12. Janssen R, van Wengen A, Hoeve MA *et al*. The same IkappaBalpha mutation in two related individuals leads to completely different clinical syndromes. *J Exp Med* 2004;**200**:559-68.
13. McDonald DR, Mooster JL, Reddy M, Bawle E, Secord E, Geha RS. Heterozygous N-terminal deletion of IkappaBalpha results in functional nuclear factor kappaB haploinsufficiency, ectodermal dysplasia, and immune deficiency. *J Allergy Clin Immunol* 2007;**120**:900-7.
14. Lopez-Granados E, Keenan JE, Kinney MC *et al*. A novel mutation in NFKBIA/IKBA results in a degradation-resistant N-truncated protein and is associated with ectodermal dysplasia with immunodeficiency. *Hum Mutat* 2008;**29**:861-8.
15. Ohnishi H, Miyata R, Suzuki T *et al*. A rapid screening method to detect autosomal-dominant ectodermal dysplasia with immune deficiency syndrome. *J Allergy Clin Immunol* 2012;**129**:578-80.
16. Courtois G. The NF-kappaB signaling pathway in human genetic diseases. *Cell Mol Life Sci* 2005;**62**:1682-91.
17. Razani B, Reichardt AD, Cheng G. Non-canonical NF-kappaB signaling activation and regulation: principles and perspectives. *Immunol Rev* 2011;**244**:44-54.
18. Yamaoka S, Courtois G, Bessia C *et al*. Complementation cloning of NEMO, a component of the IkappaB kinase complex essential for NF-kappaB activation. *Cell* 1998;**93**:1231-40.
19. Hubeau M, Ngadjeua F, Puel A *et al*. New mechanism of X-linked anhidrotic ectodermal dysplasia with immunodeficiency: impairment of ubiquitin binding despite normal folding of NEMO protein. *Blood* 2011;**118**:926-35.
20. Vinolo E, Sebban H, Chaffotte A *et al*. A point mutation in NEMO associated with anhidrotic ectodermal dysplasia with immunodeficiency pathology results in destabilization of the oligomer and reduces lipopolysaccharide- and tumor necrosis factor-mediated NF-kappa B activation. *J Biol Chem* 2006;**281**:6334-48.
21. Cordier F, Grubisha O, Traincard F, Veron M, Delepierre M, Agou F. The zinc finger of NEMO is a functional ubiquitin-binding domain. *J Biol Chem* 2009;**284**:2902-7.
22. Hadian K, Griesbach RA, Dornauer S *et al*. NF-kappaB essential modulator (NEMO) interaction with linear and lys-63 ubiquitin chains contributes to NF-kappaB activation. *J Biol Chem* 2011;**286**:26107-17.
23. Smahi A, Courtois G, Vabres P *et al*. Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. The International Incontinentia Pigmenti (IP) Consortium. *Nature* 2000;**405**:466-72.
24. Zonana J, Elder ME, Schneider LC *et al*. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am J Hum Genet* 2000;**67**:1555-62.
25. Dupuis-Girod S, Corradini N, Hadj-Rabia S *et al*. Osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency in a boy and incontinentia pigmenti in his mother. *Pediatrics* 2002;**109**:e97.
26. Darwech I, Otero J, Alhawagri M, Dai S, Abu-Amer Y. Impediment of NEMO oligomerization inhibits osteoclastogenesis and osteolysis. *J Cell Biochem* 2009;**108**:1337-45.
27. Jain A, Ma CA, Lopez-Granados E *et al*. Specific NEMO mutations impair CD40-mediated c-Rel activation and B cell terminal differentiation. *J Clin Invest* 2004;**114**:1593-602.
28. Filipe-Santos O, Bustamante J, Haverkamp MH *et al*. X-linked susceptibility to mycobacteria is caused by mutations in NEMO impairing CD40-dependent IL-12 production. *J Exp Med* 2006;**203**:1745-59.
29. Orange JS, Brodeur SR, Jain A *et al*. Deficient natural killer cell cytotoxicity in patients with IKK-gamma/NEMO mutations. *J Clin Invest* 2002;**109**:1501-9.
30. Pandey R, DeStephan CM, Madge LA, May MJ, Orange JS. NKp30 ligation induces rapid activation of the canonical NF-kappaB pathway in NK cells. *J Immunol* 2007;**179**:7385-96.
31. Tono C, Takahashi Y, Terui K *et al*. Correction of immunodeficiency associated with NEMO mutation by umbilical cord blood transplantation using a reduced-intensity conditioning regimen. *Bone Marrow Transplant* 2007;**39**:801-4.
32. Fish JD, Duerst RE, Gelfand EW, Orange JS, Bunin N. Challenges in the use of allogeneic hematopoietic SCT for ectodermal dysplasia with immune deficiency. *Bone Marrow Transplant* 2009;**43**:217-21.
33. Mizukami T, Obara M, Nishikomori R *et al*. Successful treatment with infliximab for inflammatory colitis in a patient with X-linked anhidrotic ectodermal dysplasia with immunodeficiency. *J Clin Immunol* 2012;**32**:39-49.
34. Nenci A, Becker C, Wullaert A *et al*. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* 2007;**446**:557-61.
35. Orange JS, Levy O, Brodeur SR *et al*. Human nuclear factor kappa B essential modulator mutation can result in immunodeficiency without ectodermal dysplasia. *J Allergy Clin Immunol* 2004;**114**:650-6.
36. Nishikomori R, Akutagawa H, Maruyama K *et al*. X-linked ectodermal dysplasia and immunodeficiency caused by reversion mosaicism of NEMO reveals a critical role for NEMO in human T-cell development and/or survival. *Blood* 2004;**103**:4565-72.
37. Mooster JL, Cancrini C, Simonetti A *et al*. Immune deficiency caused by impaired expression of nuclear factor-kappaB essential modifier (NEMO) because of a mutation in the 5' untranslated region of the NEMO gene. *J Allergy Clin Immunol* 2010;**126**:127-32. e7.
38. Imamura M, Kawai T, Okada S *et al*. Disseminated BCG infection mimicking metastatic nasopharyngeal carcinoma in an immunodeficient child with a novel hypomorphic NEMO mutation. *J Clin Immunol* 2011;**31**:802-10.
39. Kawai T, Nishikomori R, Izawa K *et al*. Frequent somatic mosaicism of NEMO in T cells of patients with X-linked anhidrotic ectodermal dysplasia with immunodeficiency. *Blood*. Epub 2012 Apr 19.
40. Pai SY, Levy O, Jabara HH *et al*. Allogeneic transplantation successfully corrects immune defects, but not susceptibility to colitis, in a patient with nuclear factor-kappaB essential modulator deficiency. *J Allergy Clin Immunol* 2008;**122**:1113-8. e1.
41. Cheng LE, Kanwar B, Tcheurekdjian H *et al*. Persistent systemic inflammation and atypical enterocolitis in patients with NEMO syndrome. *Clin Immunol* 2009;**132**:

124-31.

42. Permaul P, Narla A, Hornick JL, Pai SY. Allogeneic hematopoietic stem cell transplantation for X–linked ectodermal dysplasia and immunodeficiency: case report and review of outcomes. *Immunol Res* 2009;**44**:89-98.

43. Dupuis-Girod S, Cancrini C, Le Deist F *et al*. Successful allogeneic hemopoietic stem cell transplantation in a child who had anhidrotic ectodermal dysplasia with immunodeficiency. *Pediatrics* 2006;**118**:e205-11.