Diagnosis and Treatment in Anhidrotic Ectodermal Dysplasia with Immunodeficiency

Tomoki Kawai, Ryuta Nishikomori and Toshio Heike

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. The immunological features of EDA-ID include susceptibility to pathogens, impaired antibody response to polysaccharides, hypogammaglobulinemia, hyper IgM syndrome, impaired natural killer (NK) cell cytotoxicity, and autoimmune diseases. 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 signaling. 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. The second case involved a boy who suffered from multiple life-threatening infections caused by Pseudomonas aeruginosa, Mycobacterium avium, and cytomegalovirus infections. In spite of extensive searches for the cause of the refractory infections in these patients,
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
Hypomorphic mutations of NEMO impair IκBα phosphorylation such that mutant IκBα molecules accumulate in the cytoplasm, thereby inhibiting the nuclear translocation of NF-κB and subsequent activation of its target genes. 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. 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-
 Manifestations and Mutations in EDA-ID

ED1 (formerly the EDA) gene. The ectodysplasin receptor is homologous to members of the TNF receptor superfamily and is encoded by the ED1 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 IkBa impair this pathway, resulting in 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 pigmentation, (2) periorbital wrinkling and hyperpigmentation, (3) sparse absent hair, (4) hypoplastic absent sweat glands, (5) hypodontia 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 IkBa 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 RANKL-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 infectious, 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 influenzae, 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 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

Allergology International Vol 61, No2, 2012 www.jsaweb.jp/
has not been found in AL-EDA-ID patients.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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-\(\alpha\)-induced apoptosis and accounted for the severe chronic intestinal inflammation. Accordingly, the authors suggested that the impaired NF-\(\kappa\)B signalling in EDA-ID resulted in TNF-\(\alpha\) induced apoptosis and subsequent inflammatory diseases.\textsuperscript{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.\textsuperscript{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.\textsuperscript{14,18} However, since EDA is characteristic and diagnostically useful in EDA-ID patients,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{36} and in the majority of patients with incontinentia pigmenti.\textsuperscript{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.\textsuperscript{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,\textsuperscript{33,36,38} our recent study determined a much higher frequency.\textsuperscript{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.\textsuperscript{36,38}

**CLINICAL MANIFESTATIONS OF AD-EDA-ID**

Mutations of I\(\kappa\)B\(\alpha\) cause signal transduction abnormalities that are associated with NF-\(\kappa\)B activation, resulting in various clinical manifestations, analogous to mutations in NEMO.\textsuperscript{7}

In AD-EDA-ID, four mutations in the I\(\kappa\)B\(\alpha\) gene have been reported, p.Ser32Ile\textsuperscript{7,12} and three nonsense mutations p. Gln 9X, p. Trp 11X, and p.Glu14X.\textsuperscript{13-15} Among the AD-EDA-ID patients reported in the literature, EDA was a consistent finding, except in patients with I\(\kappa\)B\(\alpha\) 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 TCR\(\gamma\)7 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.\textsuperscript{7,12}

A pediatric patient with somatic mosaicism involving the p.Ser32Ile I\(\kappa\)B\(\alpha\) 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
rheumatoid-factor-positive oligoarthritis. An episodic Salmonella typhimurium infection was effectively treated with antibiotics and the patient has since been healthy.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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-

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

<table>
<thead>
<tr>
<th>Mutations of IκBα</th>
<th>p.32Ile</th>
<th>p.32Ile mosaicism</th>
<th>Gln9X</th>
<th>Trp11X</th>
<th>Glu14X</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>Severe</td>
<td>Episodic S. typhimurium infection</td>
<td>Severe</td>
<td>Recurrent pneumonia</td>
<td>Severe</td>
</tr>
<tr>
<td>Pneumocystic jiroveci infection</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cutaneous candida infection</td>
<td>+</td>
<td>Systemic JIA (in childhood)</td>
<td>Reduced (CD3, PHA)</td>
<td>Normal (PHA, Con-A)</td>
<td>Normal (PHA, PWM, Con-A, tetanus)</td>
</tr>
<tr>
<td>Autoimmune or inflammatory disease</td>
<td>-</td>
<td>RF(+) oligoarthritis (in adulthood)</td>
<td>Inflammatory bowel disease</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment</td>
<td>IVIG HSCT</td>
<td>Steroid Non-steroidal-anti-inflammatory drugs</td>
<td>IVIG HSCT (scheduled)</td>
<td>Healthy with IVIG</td>
<td>IVIG HSCT (died due to sepsis)</td>
</tr>
<tr>
<td>Gammaglobulin abnormality</td>
<td>Hypogamma-globulinemia</td>
<td>-</td>
<td>Increased IgA</td>
<td>Increased IgA, decreased IgM</td>
<td>-</td>
</tr>
<tr>
<td>Specific antibody deficiency</td>
<td>+</td>
<td>-</td>
<td>n.d.</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>Lymphocytosis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Abnormal lymphocyte proliferation</td>
<td>Normal (PHA)</td>
<td>Mildly reduced (CD3, PHA)</td>
<td>Reduced (PHA, Con-A)</td>
<td>Normal (PHA, CD3, CD28, tetanus, diphtheria)</td>
<td>Normal (PHA, PWM, Con-A, tetanus)</td>
</tr>
<tr>
<td>TCRγδ</td>
<td>Decreased</td>
<td>Normal</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Decreased</td>
</tr>
<tr>
<td>NK cell abnormalities</td>
<td>Normal NK cell activity</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Normal percent-age of NK cells</td>
<td>Reduced NK cells</td>
</tr>
<tr>
<td>Impaired TLR response</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 3  Summary of reported cases of EDA-ID in which the patient underwent HSCT

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation</th>
<th>HLA match</th>
<th>Source</th>
<th>Conditioning</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NEMO c.1167_1168 insC</td>
<td>UD 2/6 matched Disparate at HLA-A by serology, disparate at both HLA-A and both HLA-DR by DNA typing</td>
<td>CB</td>
<td>Fludarabine 150 mg/m² Melphalan 140 mg/m² rATG 12.5 mg/kg</td>
<td>Alive</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>NEMO c.1167_1168 insC</td>
<td>Matched sibling</td>
<td>PSC</td>
<td>Fludarabine 6 mg/kg/day Busulfan target 4000 μM/min i.v. × 2 days rATG 8 mg/kg</td>
<td>Graft failure</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>NEMO c.1167_1168 insC</td>
<td>Same donor</td>
<td>PSC</td>
<td>Fludarabine 5 mg/kg/day Melphalan 3.5 mg/kg Alemtuzumab 30 mg/kg</td>
<td>Alive Rash, diarrhea</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NEMO c.1167_1168 insC</td>
<td>UD 7/10 matched Disparate at HLA-B and both HLA-C</td>
<td>CB</td>
<td>Busulfan target 900-1300 μM/min i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg eATG 90 mg/kg</td>
<td>Alive</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>NEMO c.1259A&gt;C</td>
<td>UD matched</td>
<td>BM</td>
<td>Busulfan 20 mg/kg Cyclophosphamide 200 mg/kg rATG 5 mg/kg</td>
<td>Died at day +6 from veno-occlusive disease</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>NEMO c.768 + 5G&gt;A</td>
<td>UD matched</td>
<td>BM</td>
<td>Busulfan 1 mg/kg i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg rATG 9 mg/kg</td>
<td>Graft failure</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>NEMO c.768 + 5G&gt;A</td>
<td>Same donor</td>
<td>PSC</td>
<td>Fludarabine 160 mg/m²</td>
<td>Died at day +314 due to para-influenza type III virus infection</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NEMO c.458T&gt;G</td>
<td>Matched sibling</td>
<td>BM</td>
<td>Busulfan target 900-1300 μM/min i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg</td>
<td>Alive Continued colitis</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>NEMO c.931C&gt;G</td>
<td>UD Matched</td>
<td>BM</td>
<td>Fludarabine 150 mg/m² Melphalan 140 mg/m² rATG 5 mg/kg</td>
<td>Alive</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>NEMO duplication of exon 4-5</td>
<td>UD 5/8 locus matched Disparate at HLA-B by serology Disparate at HLA-A, B, and C by DNA typing</td>
<td>CB</td>
<td>Fludarabine 150 mg/m² Melphalan 140 mg/m² rATG 12.5 mg/kg</td>
<td>T-cell graft failure Died at day +60 due to sepsis</td>
<td>39</td>
</tr>
<tr>
<td>9</td>
<td>NEMO duplication of exon 4-5</td>
<td>UD 8/10 locus matched Disparate at HLA-B and C</td>
<td>CB</td>
<td>Fludarabine 5 mg/kg Cyclophosphamide 200 mg/kg rATG 9 mg/kg</td>
<td>Graft failure</td>
<td>32</td>
</tr>
<tr>
<td>9</td>
<td>NEMO duplication of exon 4-5</td>
<td>UD 7/8 locus matched Disparate at HLA-A</td>
<td>CB</td>
<td>Busulfan 1.1 mg/kg i.v. 6 h × 16 doses Cyclophosphamide 200 mg/kg Alemtuzumab 36 mg/kg</td>
<td>Graft failure Died from sepsis due to Pseudo- monas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>NEMO duplication of exon 4-5</td>
<td>Maternal haploidentical</td>
<td>BM</td>
<td>Busulfan 20 mg/kg Cyclophosphamide 200 mg/kg</td>
<td>Alive</td>
<td>43</td>
</tr>
</tbody>
</table>

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 Ixβα 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. 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. 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. 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. 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. In a case report, inflammatory colitis in an XL-EDA-ID patient was successfully treated with anti-TNFα antibody administration. 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). 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 EDAID patients have intrinsic difficulties with successful engraftment 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.

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