

## Review

# NF- $\kappa$ B-related genetic diseases

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## Abstract

The recent identification of genetic diseases (incontinentia pigmenti, anhidrotic ectodermal dysplasia with immunodeficiency and cylindromatosis) resulting from mutations affecting components of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling pathway provides a unique opportunity to understand the function of NF- $\kappa$ B *in vivo*. Besides confirming the importance of NF- $\kappa$ B in innate and acquired immunity or bone mass control, analysis of these diseases has uncovered new critical roles played by this transcription factor in the development and homeostasis of the epidermis and the proper function of lymphatic vessels. In addition, the identified mutations will help understanding at the molecular level how NF- $\kappa$ B is activated in response to cell stimulation. *Cell Death and Differentiation* (2006) 13, 843–851.

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**Keywords:** NF- $\kappa$ B; NEMO; incontinentia pigmenti, anhidrotic ectodermal dysplasia with immunodeficiency; cylindromatosis; CYLD.

**Abbreviations:** NF- $\kappa$ B, nuclear factor- $\kappa$ B; I $\kappa$ B, inhibitor of  $\kappa$ B; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; TNF-R, TNF-receptor; IL-1R, Interleukin-1 receptor; LPS, lipopolysaccharide; PGN, peptidoglycan; kB, kilobase; C-terminus, carboxy-terminus

## Introduction

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a generic name for a family of dimeric transcription factors generated by the homo- or hetero-association of members of the Rel/NF- $\kappa$ B family of proteins. This family is composed of five proteins: p50 (derived from p105 precursor), p52 (derived from p100 precursor), RelA, c-rel and RelB. In most resting cell types, NF- $\kappa$ B is kept inactive in the cytoplasm through interaction with inhibitory molecules of the I $\kappa$ B family (I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$  and I $\kappa$ B $\epsilon$ ). In response to multiple stimuli such as inflammatory cytokines, bacterial lipopolysaccharide, viral infection or stress, I $\kappa$ Bs are phosphorylated on two critical serine residues. This modification allows ubiquitination and destruc-

tion of I $\kappa$ Bs via the proteasome degradation machinery. As a consequence, free NF- $\kappa$ B enters the nucleus and activates transcription of a variety of genes participating in immune and inflammatory response, cell adhesion, growth control or protection against apoptosis.<sup>1,2</sup>

The kinase that phosphorylates I $\kappa$ B, IKK (I $\kappa$ B kinase), is a high-molecular-weight complex migrating around 700–900 kDa after gel filtration. It contains two related catalytic subunits, IKK-1/IKK- $\alpha$  and IKK-2/IKK- $\beta$ , a regulatory subunit, NEMO/IKK- $\gamma$ , and possibly other subunits such as Cdc37 and hsp90 or the recently described ELKS subunit.<sup>3–5</sup> In contrast to IKK-1 and IKK-2, NEMO exhibits no catalytic properties but cell lines defective for this protein do not activate NF- $\kappa$ B in response to many stimuli, among them tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) or lipopolysaccharide (LPS), demonstrating its key role in activation of the NF- $\kappa$ B pathway.<sup>6</sup> NEMO is supposed to provide interfaces for signaling molecules that act on IKK (Figure 1) and may participate in IKK activation through oligomerization.

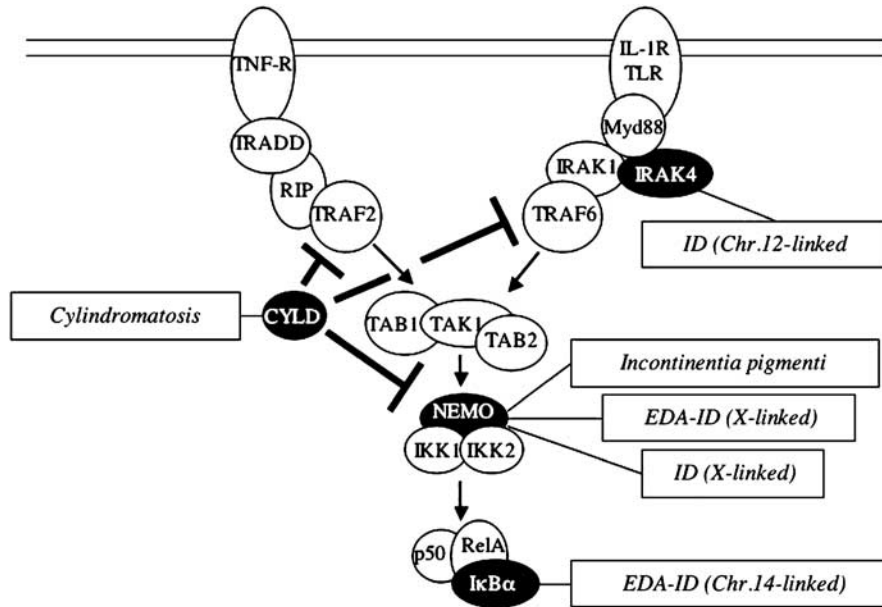
NEMO is a protein of approximately 50 kDa, which is composed of two coiled coil (CC) domains, a leucine-zipper (LZ) and a zinc finger (ZF) (Figure 2). It has been shown that NEMO interacts with IKK subunits through the N-terminal part of its CC1, whereas the C-terminal part of this domain provides a binding site for Tax or RIP.<sup>7,8</sup> In addition, the CC2/LZ part of NEMO represents the minimal oligomerization domain of the molecule.<sup>9</sup> Finally, the NEMO ZF appears required for IKK activation in response to TNF, LPS or IL-1<sup>10</sup> although its exact function remains unclear.

The gene encoding NEMO is located on the X chromosome, at Xq28, where *G6PDH* and *Haemophilia Factor VIII* genes are also present.<sup>11</sup> Such X-linkage is not observed with any other genes encoding known molecules of the NF- $\kappa$ B pathway and, as will be discussed below in more details, it has a major impact on human pathology.

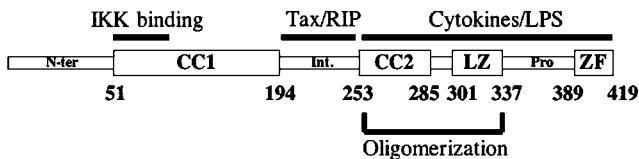
Besides the NEMO-dependent pathway of IKK activation, it has been recently demonstrated that IKK activation can also be triggered by an alternative pathway that requires the upstream kinase NIK and IKK-1 but neither NEMO nor IKK-2.<sup>12</sup> This pathway, which plays an important role in B cell development and homeostasis, does not target I $\kappa$ Bs but induces instead p100 processing to release active p50/reI $\beta$  dimers.

## NF- $\kappa$ B-Related Pathologies

Until recently, it was difficult to imagine any major human pathology caused by NF- $\kappa$ B dysfunction given the many important roles that this protein plays *in vivo*. At the best, the existence of some discrete immunodeficiency syndromes due to mutations affecting NF- $\kappa$ B subunits, such as c-rel or relB, may have been predicted. Another possibility would have been the occurrence of mutations affecting an X-linked component of the NF- $\kappa$ B pathway. This latter hypothesis



**Figure 1** TNF-R and IL-1R/TLR signaling pathways. The principal molecules participating in NF- $\kappa$ B activation by TNF-R and IL-1R/TLR signaling pathways are presented. The association of some of them (colored in black) with human pathologies is also indicated (name of the pathology and mode of transmission in boxes). See text for more details



**Figure 2** Structural and functional domains of NEMO. The sequences participating in IKK, Tax and RIP binding, NEMO oligomerization and response to pro-inflammatory cytokines or LPS are indicated. N-ter: N-terminal domain, CC: coiled coil domain, Int.: intermediate domain, LZ: leucine zipper, Pro: proline-rich domain, ZF: zinc finger

has turned out to be the right one, due to the peculiar chromosomal location of *NEMO*. Importantly, the discovery of *NEMO*-related pathologies (see below) has not only revealed unexpected roles of NF- $\kappa$ B *in vivo* but also provides a rationale for searching mutations affecting other components of the NF- $\kappa$ B signaling pathway.

### ***NEMO*-related pathologies**

#### **Incontinentia pigmenti**

Incontinentia pigmenti (IP) (OMIM # 308300) is a severe X-linked genodermatosis with an incidence of between 1/10 000 and 1/100 000, which presents almost exclusively in females, as male cases die *in utero* before the second trimester.<sup>13,14</sup> In affected females, the disorder is highly variable in presentation but always associated with skin defects. Typically, IP is characterized by four distinct dermatological stages that begin within 2 weeks after birth with blisters and an inflammatory response, accompanied by a massive eosinophilic granulocyte infiltration into the epidermis (Stage I/Vesicular Stage). Subsequently, verrucous hyperkeratotic lesions develop (Stage II/Verrucous Stage) and disappear over time, leaving

behind areas of hyperpigmentation due to melanin accumulation (Stage III/Hyperpigmented Stage). These areas, which follow the lines of Blaschko, generally disappear by the second decade (Stage IV/Atrophic Stage), but adults may still show areas of dermal scarring with lack of hair follicle.

In addition to skin signs, IP patients can also suffer from ophthalmologic, odontological or neurological problems. Ophthalmologic problems, which affect approximately 35% of patients, mostly represent abnormalities of the developing retinal vessels. Retinal detachment can be observed as a consequence of a neovascularization following retinal ischemia caused by abnormal peripheral retinal vessels. Odontological problems are characterized by delayed eruption, oligodontia, agenesis, peg-shaped or malformed teeth, supernumerary teeth and supplementary cusps and affect more than 80% of IP patients. Neurological abnormalities, observed in approximately 30% of IP cases, occur during the first weeks of life, which may concord with the neonatal cutaneous eruption, and includes epilepsy, mental retardation, hemiparesis, spasticity, microcephaly and cerebellar ataxia. In rare cases, the CNS manifestations can be fatal, when seizures leads to death due to severe vascular cerebral damages resulting in thalamic hemorrhage, ischemia and necrosis of both hemispheres. Magnetic resonance studies of IP patients exhibiting CNS abnormalities have demonstrated scattered cortical neuronal and white-matter necrosis, hypoplasia of the corpus callosum, neuronal heterotopia, cerebral atrophy and fresh hemorrhagic necrosis, with vascular congestion in the cerebral white matter.<sup>15,16</sup> It has been proposed that microvascular ischemia may be responsible for CNS abnormalities in IP.<sup>17</sup>

A very striking feature of IP pathology is the extensive X-inactivation skewing that is observed in peripheral blood cells of female patients. This skewing reflects an efficient mechan-

ism of counter-selection against cells expressing the mutated X chromosome. It is also potent in other cell types, such as the hepatocyte. Indeed, a case of female hemophilia has been reported resulting from the marriage between an IP patient and an hemophilic.<sup>18</sup> As will be explained below, such extensive skewing does not take place in the antenatal epidermis.

The gene responsible for IP was originally mapped to an interval of about 2 Mb distal to the colour vision locus in Xq28. Among the putative candidate genes was *NEMO*. It was among the first genes to be sequenced because of an apparent increased sensitivity of IP embryonic fibroblasts to apoptosis. Quite remarkably, the analysis of a large collection of patients showed that 70–80% of them carried the same complex rearrangement of the *NEMO* locus.<sup>19</sup> This rearrangement induces excision of the region between two MER67B repeated sequences located upstream of exon 4 and downstream of exon 10, respectively (Figure 3). It may be associated with the presence of a *NEMO* pseudogene ( $\Delta$ *NEMO*) located 22 kb 3' apart from *NEMO* in a reverse orientation.<sup>20</sup> Indeed, both *NEMO* and  $\Delta$ *NEMO*, which contains sequence highly homologous (more than 99%) to *NEMO* exons 4–10, appear inserted into a similar genomic domain of approximately 35.5 kb that has arisen by duplication 10–15 million years ago.

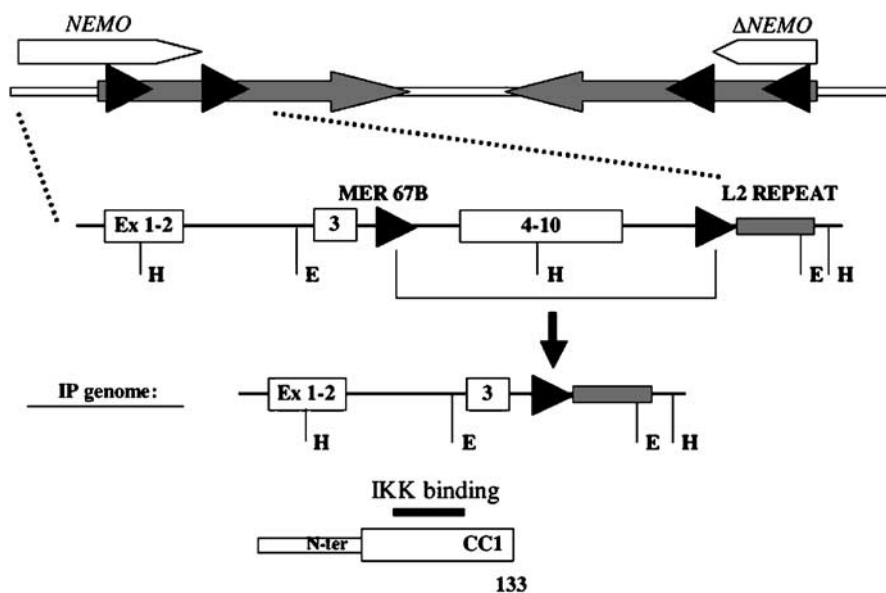
The recurrent *NEMO* rearrangement results in the synthesis of a truncated 133 amino acids protein (corresponding to exons 1–3), which is devoid of activity but still able to interact with the IKKs. A lack of NF- $\kappa$ B activation in IP patients carrying this rearrangement was demonstrated by studying fetus-derived primary fibroblasts: these cells are unresponsive to all tested NF- $\kappa$ B-activating stimuli, they do not show degradation of the I $\kappa$ B molecules when stimulated, and they are very sensitive to TNF-induced apoptosis.<sup>19</sup>

Besides the *NEMO* rearrangement that affects most IP patients, other *NEMO* mutations have been identified.<sup>19,21,22</sup> Although many of them, represented by non-sense or frame-shift mutations, result in large truncations of *NEMO* several missense mutations have also been reported. Their precise effect on NF- $\kappa$ B signaling will deserve further studies since, being associated with a severe pathology, they may provide valuable information regarding *NEMO* function (see related comment in the 'EDA-ID' section).

Although the vast majority of IP patients are females, a small collection of males exhibiting all the signs of the disease has also been identified. Genetically, such cases may be explained by the presence of the klinefelter syndrome (47, XXY), a karyotype compatible with survival, or early somatic mutation of the *NEMO* gene. Molecular studies of three IP boys with normal karyotype have demonstrated that they had both wild type and deleted copies of *NEMO* and were therefore mosaic for the common DNA rearrangement.<sup>23</sup>

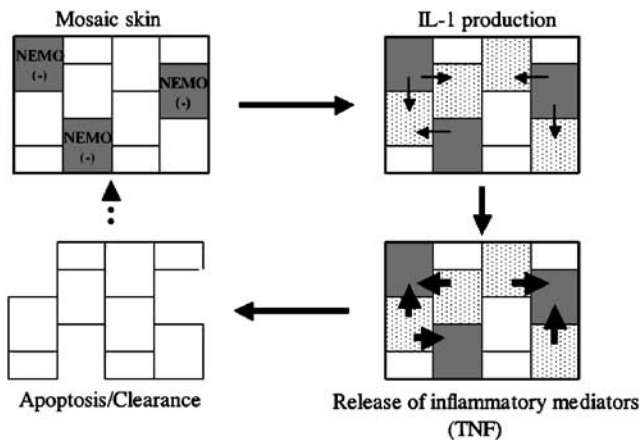
Understanding IP dermatosis remains a major challenge. Indeed, the skin phenotype of IP patient is quite difficult to interpret due to the complex interplay that exists in this tissue between cells carrying a normal copy of *NEMO* and those carrying a defective one. As said above, IP females exhibit extremely skewed X-inactivation in blood cells, resulting from a counter-selection of cells expressing the mutated *NEMO* locus. For some unknown reasons, X inactivation skewing is less complete in the skin and selective elimination of cells bearing a mutated X chromosome only starts at birth. This event appears directly responsible for the dermatosis observed in IP patients.

Through the analysis of mouse models of IP (*NEMO* and skin-specific IKK-2 KO mice, see Pasparakis *et al.* in this issue for a comprehensive review), a putative sequence of molecular and cellular events associated with IP dermatosis can be proposed (Figure 4). As said above, IP patients



**Figure 3** Genomic rearrangement of *NEMO* in IP. The 35.5 kb genomic duplication that contains *NEMO* and  $\Delta$ *NEMO* is represented by a gray arrow. To simplify the picture, exons 1 and 2 and 4–10 of *NEMO* are represented intronless. The hypothetical *NEMO* protein that would be produced after the genomic rearrangement is also shown

present at birth with a mosaic skin composed of cells expressing, due to lyonization, either wt or mutated *NEMO*. In response to some unknown intrinsic or extrinsic signal(s) mutated cells start to produce, or help producing by surrounding wild-type cells, proinflammatory cytokines such as IL-1, a well-known stress-response molecule of the epidermis. This, in turn, appears to induce the release of TNF by wild-type cells, which acts back by inducing hyperproliferation and inflammation of wild-type cells and apoptosis of mutated cells. The whole process results in elimination of the mutated cells and, consequently, disappearance over time of the skin lesions. In this hypothetical model, the mutated cells initiating the process are therefore



**Figure 4** Molecular and cellular events associated with IP dermatosis. A putative sequence of events triggering the elimination of *NEMO* (-) cells in the epidermis is presented based on the analysis of mouse models of IP (see text for details). The broken arrow indicates that the whole process can restart if *NEMO* (-) cells are not completely eliminated after the first round

indirectly responsible for their own elimination. Interestingly, if all the mutated cells are not cleared off this way during childhood, they may trigger again the whole process later on. This is something that has been indeed observed in few IP patients.<sup>24,25</sup>

#### Anhidrotic ectodermal dysplasia with immunodeficiency (X-linked form)

Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) (OMIM# 300291) is a rare and complex X-linked pathology exclusively affecting males.<sup>26–29</sup> It combines a severe sensitivity to infection with abnormal development of skin adnexes (hair follicles, sweat glands and teeth). The mode of genetic transmission, a perturbed immune response and some similarities with IP led to the analysis of the *NEMO* gene in several EDA-ID patients. Most of them indeed carry mutations in *NEMO* but instead of leading to large truncations of the *NEMO* molecule as observed in IP, the mutations are mostly missense mutations or small deletions only affecting the ZF (Table 1).<sup>30–34</sup> Their molecular characterization is likely to provide valuable insights into *NEMO* function but for only few of them some specific defect has been identified.

Importantly, all the EDA-ID mutations lead to reduced but not abolished NF- $\kappa$ B activation, explaining why affected male patients survive. Moreover, since their single X chromosome carries the mutated gene, the physiological consequences of NF- $\kappa$ B dysfunction in humans can be directly observed. In contrast, female patients carrying the same *NEMO* mutations remain healthy or exhibit very mild signs of IP, depending on the kind of mutation and X-inactivation pattern. Recently, an IP female carrying a new mutation in the *NEMO* gene (insA790) was described with immunodeficiency.<sup>35</sup> In this case it was observed a late progressive selection against peripheral blood cells carrying mutated X-chromosome.

**Table 1** Missense, nonsense and internal deletions of *NEMO* in pathology

Mutation	Domain	Pathology	Molecular defect	Reference
$\Delta(1-37)$	N-ter	ID	ND	59
E57K	CC1	IP	ND	19,22
R62X	CC1	IP	Very short inactive polypeptide	19
$\Delta$ K90	CC1	IP	Impaired interaction with IKK-2	22
R123W	CC1	IP	ND	22
L153R	CC1	EDA-ID	ND	34
R175P	CC1	EDA-ID	ND	31
L227P	Inter.	EDA-ID	ND	31
Q239X	Inter.	IP	No regulatory/oligomerization C-ter domain	22
R254G	Inter.	ID	ND	60
A288G	CC2	EDA-ID	Impaired oligomerization <sup>a</sup>	31
D311N	CC2/LZ	EDA-ID	ND	31
$\Delta(351-373)$	Pro-rich	ID	ND	58
Q384X	Pro-rich	IP	No ZF	22
E391X	Pro-rich	EDA-ID	No ZF	30
Q403X	ZF	EDA-ID	Truncated ZF	34
D406V	ZF	EDA-ID	ND	33
M407V	ZF	IP	ND	19
C417F	ZF	EDA-ID	ND	31
C417R	ZF	EDA-ID	ND	31,33,34
C417Y	ZF	EDA-ID	ND	34
X420W	C-ter	OL-EDA-ID	Extra aa/severe protein instability	19,31

N-ter: N-terminus domain; CC1: coiled coil #1; Inter.: intermediate domain; CC2: coiled coil #2; LZ: Leucine zipper; Pro-rich: Proline-rich domain; ZF: zinc finger; C-ter: C-terminus (see Figure 1); ID: immunodeficiency; ND: not determined; aa: amino acids. <sup>a</sup>Vinolo *et al.* (submitted).

When the X-inactivation was completely skewed, all immunodeficiency signs disappeared.

The immunodeficiency affecting male patients with EDA-ID is characterized by unusually severe life-threatening and recurrent bacterial infections of lower respiratory tract, skin, soft tissues, bones and gastrointestinal tract, as well as meningitis and septicemia in early childhood. The causative pathogens are most often Gram-positive bacteria (*S. pneumoniae* and *S. aureus*), followed by Gram-negative bacteria (*Pseudomonas* spp. and *Haemophilus influenzae*) and mycobacteria.

The high sensitivity of EDA-ID patients to infection results from an impaired cellular response of peripheral blood lymphocytes to LPS, IL-1 $\beta$ , IL-18, TNF- $\alpha$  and CD40L. Other NF- $\kappa$ B-dependent pathways are likely to be affected as well. Indeed, NEMO-dependent NF- $\kappa$ B activation is important for the signaling pathways downstream of Toll receptors (Tlr) and these receptors represent major pathogen sensors.<sup>36</sup>

A consistent feature of EDA-ID pathology is impaired antibody response to polysaccharide antigens. Most patients also exhibit hypogammaglobulinaemia with low serum IgG levels. The levels of other immunoglobulin isotypes (IgA, IgM and IgE) can vary but numerous EDA-ID patients have been described with elevated serum IgM levels (The so-called 'hyper-IgM' phenotype). This syndrome can be caused by the inability of their B cells to switch in response to CD40 ligand (CD40L). Alternatively, the immunoglobulin switch can be normal but defective proliferation and differentiation generates an 'hyper-IgM-like' phenotype.

In addition to these B-cell abnormalities, impaired NK activity has also been reported in several patients.<sup>37</sup> In contrast, patients with EDA-ID have a normal T-cell proliferation index in response to mitogens and antigens.

The quite enigmatic association of immunodeficiency with anhidrotic ectodermal dysplasia in EDA-ID patients has revealed an unexpected contribution of the NF- $\kappa$ B signaling pathway to the development of skin appendages. Rapid progress have been made recently concerning the molecular process that ensure proper morphogenesis of epidermis adnexes and this now allows to understand how NF- $\kappa$ B may operate at this level.

Anhidrotic ectodermal dysplasia (EDA) is a well-known genetic disease<sup>38</sup> that can result from mutations affecting any of three loci in humans as well as in mice. The first identified locus, called *tabby* in mice, is located on the X chromosome, and codes for a member of the TNF family, ectodysplasin/EDA-A1,<sup>39</sup> a membrane-associated protein produced in cell types/tissues of ectodermal origin, such as keratinocytes, hair follicles and sweat glands.<sup>40</sup> Ectodysplasin is a type II transmembrane protein<sup>41,42</sup> that is released as a trimer in the extracellular compartment after furin-dependent cleavage.<sup>43</sup>

The second locus responsible for EDA, called *downless* in mice, is located on chromosome 2 in humans and accounts for both autosomal recessive and dominant types of the disease.<sup>44,45</sup> It encodes a death-domain containing member of the TNF-R family, EDAR, and is a specific receptor for ectodysplasin.<sup>46,47</sup> Its expression is restricted to placodes, thickenings of epithelia where epidermal appendages begin to form.<sup>48,49</sup>

The third locus whose disruption leads to EDA has been identified only recently in *crinkled* mice and subsequently found mutated in a human family.<sup>50,51</sup> It is located on chromosome 1 in humans (chromosome 13 in mouse) and encodes a death domain containing adaptor protein, EDAR-ADD/CR, which binds EDAR through a homotypic death domain interaction. EDARADD is also able to interact with the adaptor molecule TRAF2.

Since members of the TNF/TNF-R families are very often connected to NF- $\kappa$ B signaling, the EDA syndrome caused by *NEMO* mutations in EDA-ID can be explained. Additional biochemical studies have confirmed that ectodysplasin/EDAR interaction indeed results in NF- $\kappa$ B activation.<sup>31,47,52</sup> Therefore, mutations in three genes that encode members of a signaling cascade that leads to NF- $\kappa$ B activation result in anhidrotic ectodermal dysplasia. Although the details of the ectodysplasin/EDAR signaling pathway are not fully characterized, it is clear from analyzing the mouse models of EDA pathology that this pathway is involved very early during development of hair follicle morphogenesis,<sup>44</sup> assigning a previously unrecognized role for NF- $\kappa$ B in this process.

#### EDA-ID with osteopetrosis and lymphoedema (OL-EDA-ID)

A variant form of EDA-ID (OL-EDA-ID) exhibiting two additional defects, osteopetrosis and lymphoedema, has been described in two distinct patients.<sup>19,31,53</sup> Osteopetrosis, which results from defective bone resorption by osteoclasts, can be easily associated with NF- $\kappa$ B since it has been shown that p50/p52KO mice exhibit osteopetrosis and that the NF- $\kappa$ B-dependent RANK pathway plays an essential role in osteoclast function.<sup>54–56</sup> Primary lymphoedema, a dysfunction specifically affecting lymphatic vessels, is still poorly understood at the genetic/biochemical level. Nevertheless, the gene causing familial lymphoedema, *V-EGFR-3*, has been identified and overexpression of its product appear to results in NF- $\kappa$ B activation.<sup>57</sup>

Remarkably, the two reported OL-EDA-ID patients were found to carry the same genetic defect: the replacement of the *NEMO* stop codon with tryptophan, leading to the addition of 27 irrelevant amino acids at the C-terminus of the molecule.<sup>19,31</sup> This seemingly benign mutation actually turned out to be lethal, at least because it strongly destabilizes the *NEMO* protein and lead to almost undetectable levels of this molecule.

#### Immunodeficiencies without EDA

More recently, immunodeficiencies not associated with EDA have been reported to be caused by *NEMO* mutations. In the first case, the patient exhibited infections during childhood and subsequently developed an atypical mycobacteria infection. Several impaired immunologic functions were detected, among them reduced CD40-induced cell proliferation and variable TLR-induced TNF- $\alpha$  production. These defects were linked to a splice site mutation affecting exon 9 of *NEMO* with variable penetrance.<sup>58</sup> The second described patient experienced multiple infections (*M. avium*, *H. influenzae*, *S. pneumoniae*) leading to osteomyelitis, dermatitis and

bronchiectasis. He presented with an hyper-IgM phenotype and low IFN- $\gamma$  synthesis by blood cells. The identified *NEMO* mutation in this case may result in synthesis of a protein lacking the first 37 amino acids.<sup>59</sup> How such defect may impact upon NEMO function is not known.

Finally, a missense *NEMO* mutation (R254G) have been identified causing a quite unorthodox phenotype, mostly characterized by idiopathic CD4 lymphopenia and chronic disseminated *M. avium* infection involving the lung, lymph nodes and bone marrow.<sup>60</sup>

NEMO-related pathologies provide a very fascinating example of the complex genotype–phenotype relationship that may result from the interplay between chromosome X location of a gene, its function and the various mutations that it may carry. The X-linkage of *NEMO* appears to act not only as a natural screen that helps discarding the most severely defective mutations but it may also indirectly favor the persistence in the body (or the general human population) of hypomorphic mutations that can exert deleterious effects later on or in a discrete subset of tissues. Therefore, the carrier female problem, which is a well known recurrent problem in human genetics, appears here vastly amplified by the role that NF- $\kappa$ B plays in many distinct physiological reactions. From this, it can be predicted that the wide spectrum of dysfunctions already caused by *NEMO* mutations will be further expanded in the future.

### *I $\kappa$ B $\alpha$* -related pathology

#### EDA-ID (autosomal dominant form)

So far, between 20 and 30 patients have been reported worldwide as suffering from EDA-ID. Most of them exhibit *NEMO* mutations but not all, suggesting that other loci, most likely related to NF- $\kappa$ B, may also be responsible for the disease.

Very recently, two independently analyzed patients exhibiting a similar phenotype have been described as mutated in one of the two copies of the gene encoding *I $\kappa$ B $\alpha$* .<sup>61,62</sup> This new autosomal dominant form of EDA-ID shares many similarities with *NEMO*-related EDA-ID, but is also characterized by an unusual feature. It is associated with severely impaired T cell proliferation, something that is not observed with *NEMO* mutated patients. As a consequence, recall antigens are not able to generate an effective response and memory T cells are absent.

In both cases, an heterozygous mutation affecting Ser32, one of the two phospho-acceptor sites of *I $\kappa$ B $\alpha$* , has been identified. Accordingly, the NF- $\kappa$ B dimers that are under *I $\kappa$ B $\alpha$*  control cannot be activated upon cell stimulation because of a lack of *I $\kappa$ B $\alpha$*  degradation. This is not the case for other dimers that are associated with *I $\kappa$ B $\beta$*  and *I $\kappa$ B $\epsilon$* , probably explaining, at least in part, the difference of phenotypes between *NEMO*-related and *I $\kappa$ B $\alpha$* -related-EDA-ID.

### *CYLD*-related pathologies

#### Familial cylindromatosis and multiple familial trichoepithelioma

Familial cylindromatosis/Spiegler–Brooke syndrome (OMIM# 132700) is an autosomal dominant disease characterized by

benign tumors (cylindromas) appearing during adulthood on hairy part of the body including the scalp.<sup>63</sup> Cylindromas are exclusively derived from skin appendages such as eccrine and apocrine sweat glands and are supposed to develop from a stem cell compartment, the folliculosebaceous-apocrine unit that gives rise to various skin adnexes. The gene causing cylindromatosis, *CYLD*, has been recently identified and appears to encode a tissue-specific tumor suppressor.<sup>63</sup>

Interestingly, inside the same family of cylindromatosis patients, some affected members can also exhibit hair follicle tumors (trichoepitheliomas) intermingled with cylindromas.<sup>64</sup> Since multiple familial trichoepithelioma (MFT, OMIM# 601606) is itself a well-recognized genetic disease, sequencing of the *CYLD* gene have been carried out. In several distinct MFT families, *CYLD* mutations have been identified, demonstrating that both cylindromatosis and MFT share an identical genetic basis.<sup>65–67</sup> From these observations, it can be predicted that other less well-known pathologies characterized by various adnexal neoplasms may also be caused by *CYLD* mutations.

A functional relationship between *CYLD* and NF- $\kappa$ B has been recently uncovered based on two different sets of study. In the first one, the use of two-hybrid screenings in yeast, with *NEMO* as a bait, allowed the isolation of *CYLD*.<sup>68,69</sup> Besides binding to *NEMO*, *CYLD* has also been shown to interact with TRAF2 and TRIP.<sup>68–70</sup> In another study, *CYLD* has been recovered following a whole-scale search aimed at identifying members of the deubiquitinase family controlling NF- $\kappa$ B activation by TNF- $\alpha$ .<sup>71</sup>

Upon overexpression, *CYLD* has been shown to negatively regulate NF- $\kappa$ B activation induced by TNF- $\alpha$ , IL-1- $\beta$  or CD40 and its deubiquitinase activity is required for this function.<sup>68,69</sup> Interestingly, it has been shown that IKK activation involves an ubiquitination event possibly targeting TRAFs, TAB2 and TAB3 (regulatory subunits of TAK1 kinase, a component of the TNF- $\alpha$  and IL1/TLR signaling pathways) or *NEMO*.<sup>72</sup> Polyubiquitinated chains that are added to these putative substrates require Lysine 63 (K63) of ubiquitin but not Lysine 48, indicating that they do not act by providing a tag for protein degradation through the proteasome. *CYLD*, which exhibits a restricted deubiquitinase specificity towards K63 chains, is supposed to regulate the IKK activation process at this level.

Most mutations found in familial cylindromatosis or familial multiple trichoepithelioma are C-terminal deletions that affect the catalytic domain of *CYLD*. *CYLD* molecules exhibiting this class of mutations are unable to negatively regulate NF- $\kappa$ B activation, suggesting that cylindromatosis is directly linked to perturbed NF- $\kappa$ B signaling. To explain how this defect may relate to the disease, it has been proposed that excessive protection against apoptosis resulting from uncontrolled up-regulation of NF- $\kappa$ B participates in the genesis of cylindromas. Attempts at blocking NF- $\kappa$ B activation and enhanced apoptosis resistance caused by *CYLD* dysfunction have been reported.<sup>71</sup> Nevertheless, it remains uncertain whether the use of NF- $\kappa$ B inhibitors will improve the condition of cylindromatosis patients. As said above, the exact *in vivo* targets of *CYLD* are not completely identified and besides acting on *NEMO* *CYLD* may also control the ubiquitination of TRAFs or associated partners. Cylindroma formation might therefore reflect a combination of

defective signaling pathways. Among the signaling pathways that may also be defective in cylindromatosis is the JNK signaling pathway. It has been recently shown to be under CYLD control, via the effect of this deubiquitinase on TRAF2 or TRAF6.<sup>73</sup> Therefore, cylindromatosis is unlikely to be a 'pure' NF- $\kappa$ B-related pathology.

### IRAK-4-related pathology

IRAK-4 is a member of the IRAK family of protein kinases that plays an essential role in IL-1R and TLR signaling pathways.<sup>74</sup> It interacts with both Myd88 and IRAK-1 and its catalytic activity is required for IRAK-1 activation (Figure 1). Once hyperphosphorylated, IRAK-1 associates with TRAF6, triggering activation of NF- $\kappa$ B and MAPKs pathways.

Several patients exhibiting recurrent bacterial infections, especially caused by extracellular pyogenic bacteria such as *Streptococcus pneumoniae* or *Staphylococcus aureus*, were identified as carriers of *IRAK-4* mutations.<sup>75,76</sup> Three patients were shown to be homozygous for the mutation whereas the fourth one exhibited a 'compound heterozygous' phenotype with each chromosome exhibiting a distinct *IRAK-4* mutation. In all cases, the mutation results in truncation of the kinase domain of IRAK-4 and should produce an inactive protein. Nevertheless, Western blot analysis of cell extracts derived from the patients did not detect any protein due to the absence of *IRAK-4* mRNA. This is probably due to the clearance of mutated mRNAs by nonsense mediated decay.

As predicted from what is known about IRAK-4 function, cells from mutated *IRAK-4* patients were shown to be unresponsive to a large set of stimuli using receptors of the IL-1R/TLR families. A defective response was observed with IL-1, IL-18 and various TLR ligands, such as LPS, peptidoglycan (PGN), zymosan or flagelin whereas response to TNF remained unaffected. When activation of NF- $\kappa$ B and p38 signaling pathways was assessed, a defect was observed in both cases. This suggests that immodeficiency caused by *IRAK-4* mutations is not exclusively linked to defective NF- $\kappa$ B signaling but may also involve perturbed MAPKs signaling.

The recent identification of genetic diseases caused by impaired NF- $\kappa$ B signaling have provided valuable information regarding the *in vivo* function of this very important signaling pathway. Besides confirming the critical role that NF- $\kappa$ B plays in innate and acquired immunity or in bone mass control, analysis of diseases such as IP and EDA-ID have also revealed a previously unsuspected participation of NF- $\kappa$ B in skin homeostasis, both in the interfollicular and follicular compartments, or in lymphatic vessels development. In addition, identification of pathogenic *NEMO* or *CYLD* mutations, especially the missense ones, should allow a more precise understanding of how each molecule works inside the cell to regulate positively or negatively the IKK activation process. Finally, the discovery that NF- $\kappa$ B-related human genetic diseases indeed exist will broaden up the spectrum of putative candidate genes/mutations that should be analyzed when trying to identify the cause of an immunodeficiency or a skin-related pathology.

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