Rare mendelian primary immunodeficiency diseases associated with impaired NF-κB signaling

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Mendelian primary immunodeficiency diseases (MPIDs) are rare disorders affecting distinct constituents of the innate and adaptive immune system. Although they are genetically heterogeneous, a substantial group of MPIDs is due to mutations in genes affecting the nuclear factor-κB (NF-κB) transcription pathway, essential for cell proliferation and cell survival and involved in innate immunity and inflammation. Many of these genes encode for crucial regulatory components of the NF-κB pathway and their mutations are associated with immunological and developmental signs somehow overlapping in patients with MPIDs. At present, nine different MPIDs listed in the online mendelian inheritance in man (OMIM) are caused by mutations in at least nine different genes strictly involved in the NF-κB pathway that result in defects in immune responses. Here we report on the distinct function of each causative gene, on the impaired NF-κB step and more in general on the molecular mechanisms underlining the pathogenesis of the disease. Overall, the MPIDs affecting the NF-κB signalosome require a careful integrated diagnosis and appropriate genetic tests to be molecularly identified. Their discovery at an ever-increasing rate will help establish a common therapeutic strategy for a subclass of immunodeficient patients.

INTRODUCTION

The nuclear factor-κB (NF-κB) family of transcription factors regulates diverse biological processes, including many aspects of immunological functions. Both innate and adaptive immune responses as well as the development and maintenance of the cells and tissues that comprise the immune system are, at multiple steps, under the control of the NF-κB family of transcription factors. The NF-κB family includes the structurally homologous transcription factors NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and c-Rel (Figure 1). These transcription factors share a Rel homology domain necessary for DNA binding, dimerization and interaction with the inhibitor. They can form homo- and hetero-dimers and can bind to a variety of related target DNA sequences called κB sites to modulate gene expression. The p65, RelB and c-Rel proteins contain C-terminal transcription activation domains (TADs) that enable co-activator recruitment and target gene expression (Figure 1). As p50 and p52 lack TADs, they can activate transcription by forming heterodimers with p65, RelB or c-Rel, or by recruiting other TAD-containing proteins. However, as homodimers lacking the ability to drive transcription, they can repress transcription through the binding to DNA.

In resting cells, NF-κB dimers are retained in the cytoplasm by the inhibitor of NF-κB proteins (IκB), which consist of IκBα, IκBβ, IκBγ, IκBε, Bcl3, IκBC, p100, p105, IκBS and, recently, IκBN (Figure 1). All known IκB proteins contain multiple ankyrin repeats that mediate the association between IκB and NF-κB dimers. The typical IκBα, -β and -ε molecules contain six ankyrin repeats, whereas the other IκBαs contain seven or eight repeats (Figure 1). The function of the IκB proteins is to prevent the NF-κB DNA binding: the ankyrin repeats interact with the Rel homology domain of the NF-κB proteins, thus masking their nuclear localization sequence and preventing nuclear translocation. The release of NF-κB dimers from the IκB proteins depends on the activation of the IκB kinase (IKK) complex, which consists of two catalytically active kinases, IκB kinase-β (IKKβ) also called IKK2) and IκB kinase-α (IKKα also called IKK1), and of the regulatory subunit NF-κB essential modulator (NEMO, also called IKKγ; Figure 2).

A wide range of stimuli, including lipopolysaccharides, interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), cause the activation of the IKK complex, which leads to the phosphorylation of the IκB proteins (e.g., IκBα at Ser32 and Ser36, and IκBγ at Ser19 and Ser23). The phosphorylated IκB proteins are subsequently ubiquitinated and degraded, allowing the nuclear translocation of NF-κB and the activation of target gene transcription (Figure 3).

Although the activity of NF-κB is regulated by nuclear translocation, its transcriptional activity is further regulated by post-translational modifications. These regulatory modifications, including phosphorylation, ubiquitination, nitrosylation and acetylation, have distinct functional consequences and have a key role in determining the duration and strength of NF-κB nuclear activity as well as its transcriptional output. For example, the acetylation of p65 at K218 and K221 inhibits IκBα binding and enhances DNA binding, whereas the acetylation of p65 at K122 and K123 inhibits its transcriptional activating activity.

An alternative pathway leading to NF-κB activation called the non-canonical pathway also exists, which depends on the IKKα-mediated phosphorylation of p100 associated with RelB.
The activation of p100/RelB complexes occurs during the development of lymphoid organs responsible for the generation of B and T lymphocytes. Only a small number of stimuli are known to activate NF-κB via this pathway, and these factors include lymphotoxin B and B-cell-activating factor. This pathway utilizes a complex consisting of two IKKα subunits, but not NEMO. Ligand-induced activation results in the activation of NF-κB-inducing kinase, which phosphorylates and activates the IKKα complex, which in turn phosphorylates p100, leading to the processing and liberation of the p52/RelB active heterodimer. Impaired NF-κB activation due to the identified genetic alterations in molecules involved in the NF-κB pathway is responsible for some types of MPIDs. Here, we report an overview on MPIDs due to mutations in components proximally linked to the NF-κB activation pathway that result in defects in immune responses, providing a better understanding of the mechanisms underlying these disorders.
information about the impact of each mutation on the impairment of NF-κB.

AD-EDA-ID
Autosomal dominant ectodermal dysplasia with immunodeficiency (AD-EDA-ID) (OMIM 612132) is a rare primary immunodeficiency associated with ectodermal dysplasia, due to heterozygous mutations of the NFKBIA gene, localized on chromosome 14 and encoding the inhibitory protein of the NF-κB pathway, IkBα (Figure 3; Table 1). This mutation abrogates the phosphorylation of IkBα Ser32, required for the ubiquitination and proteasomal degradation of IkBα. Other IkBα mutations, p.Gln9X, p.Glu14X and p.Trp11X, cause a premature termination of protein translation and a restart from Met37 of IkBα, resulting in a IkBα protein that is N-terminally truncated and lacks both of the critical serine residues, Ser32 and Ser36. The p.Ser32Tyr mutation results in a defective IkBα protein that is incapable of blocking NF-κB activation owing to the gain-of-function of the IkBα protein.

XL-EDA-ID
X-linked anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID, OMIM 300291) is a rare primary immunodeficiency associated with a developmental disorder due to mutations in the X-linked gene named NF-κB essential modulator (NEMO) also called IkBKG that encodes for the regulatory subunit of the IKK complex (Figure 2), essential for the canonical activation of NF-κB (Figure 3, Table 1). This event causes the nuclear translocation of NF-κB and subsequent activation of its target genes.

Immunological aspects
The broad immunological phenotypes of XL-EDA-ID patients are responsible for their susceptibility to infections with invasive pyogenic bacteria (meningitis, sepsis, arthritis, osteomyelitis and abscesses), environmental mycobacteria, and to a lesser extent, parasites, viruses and fungi. The patients suffer from a profound combined immunodeficiency, and, to a lesser extent, parasites, viruses and fungi. Moreover, the patients have low proportions of memory CD4 and CD8 T cells and no hypogammaglobulinemia with no specific antibodies; some also have low proportions of memory CD4 and CD8 T cells and no T-cell receptor γ/δ T cells and display a severe impairment of T-cell proliferation in response to anti-CD3.

Genetic aspects
Six heterozygous mutations in the NFKBIA gene have been identified in AD-EDA-ID patients. The pathogenic mutations have a dominant effect and they are called ‘hypermorphic mutations’ because they enhance the inhibitory capacity of IkBα impairing the phosphorylation and degradation of IkBα and resulting in the partial retention of NF-κB dimers in the cytoplasm.

Molecular aspects
The IkBα protein, a member of the serine/threonine protein kinase family, contains phosphorylation sites at its N-terminal, ankyrin repeat domains (Figure 1) in its central portion, and, at its C-terminal, a repeated peptidic sequence rich in proline, glutamic acids, serine and threonine domains. IkBα inhibits the activation of NF-κB, whereas its phosphorylation at the level of Ser32 and Ser36 triggers IkBα ubiquitination, leading to proteasomal degradation (Figure 3). This event causes the nuclear translocation of NF-κB and subsequent activation of its target genes.

The first case of AD-EDA-ID in which a p.Ser32Ile mutation was reported was identified by Courtois et al. This mutation abrogates the phosphorylation of IkBα Ser32, required for the ubiquitination and proteasomal degradation of IkBα. Other IkBα mutations, p.Gln9X, p.Glu14X and p.Trp11X, cause a premature termination of protein translation and a restart from Met37 of IkBα, resulting in a IkBα protein that is N-terminally truncated and lacks both of the critical serine residues, Ser32 and Ser36. The p.Ser36Tyr mutation results in a defective IkBα degradation and impaired NF-κB activation. As well as p.Met37Lys, it is capable of blocking NF-κB activation owing to the gain-of-function of the IkBα protein.
to the NEMO mutations are characterized by the following: a dysregulated immunoglobulin synthesis or hyper-immunoglobulin M syndrome; a defective antipolsaccharide antibody synthesis (antipneumococcal antibody and isohemagglutinin); reduced LPS and IL-1 family protein responses; and defective natural killer cell activity.10-22 Recently, a genotype/phenotype correlation was identified.23

Genetic aspects
All patients with XL-EDA-ID are male. The first NEMO mutations impairing NF-kB activation in XL-EDA-ID patients were described in 200024 and 200125. Up to 100 male patients with about 43 different mutations of NEMO have been reported.26 The NEMO mutations in XL-EDA-ID patients are defined 'hypomorphic' because they lead to an impairment of NF-kB signaling, but not to its abolition.27 Indeed, the NEMO loss-of-function mutations are lethal for males in utero.28,29

Molecular aspects
The NEMO protein consists essentially of a series of domains: Coiled-coil (CC) 1 in the N-terminal segment; helix-loop-helix 2 (HLH2) in the middle segment; and the CC2-leucine zipper (LZ) regulatory domain in the C-terminal segment. NEMO also has a zinc-finger (ZF) domain at its N-terminal end (Figure 2).30 The function of NEMO depends on its dimerization and its ability to interact with linear or K63-linked polyubiquitin chains.31-34 This function requires the CC2-LZ domain, which is involved in NEMO dimerization and contains a ubiquitin-binding site called NEMO-optineurin-ABIN ubiquitin-binding in ABIN and NEMO/ABIN-ubiquitin binding, and the LZ domain, which bears a second ubiquitin-binding site.35,36

The degree of impairment of the NF-kB pathway depends on the NEMO mutated domain.26,27 The mechanisms by which some mutations associated with XL-EDA-ID affect NEMO's structural and functional integrity have been investigated. The p.Ala288Gly mutation, which affects the CC2 domain, has no effect on the protein level but destabilizes the NEMO oligomers, altering the assembly of the IkB kinase complex and consequently impairing the canonical activation of NF-kB.37 The p.Asp311Asn and p.Asp311Gly mutations on the NEMO-optineurin-ABIN ubiquitin-binding site of NEMO impair NEMO-ubiquitin binding, with no detectable effect on NEMO expression and folding.38 p.Glu315Ala and p.Arg319Gln, which affect the LZ domain, disrupt the formation of the salt bridge normally formed between residues Glu315 and Arg319 without affecting NEMO protein production.39,40 Moreover, the folding defect of the p.Glu315Ala mutant is responsible for the defect in binding to the ubiquitin chains.41 The p.Cys417Phe substitution modifies the structure of the C-terminal end of the ZF α-helix and decreases its stability, which leads to a defect in NF-kB activation. On the other hand, p.Cys417Arg does not affect the expression of the NEMO protein but impairs c-Rel activation in response to CD40 ligation.42 Moreover, mutations in the ZF domain are very common and are associated with some of the more severe phenotypes (e.g., ectodermal dysplasia with immune deficiency and osteopetrosis).

**AUTOSOMAL RECESSIVE IKK2 DEFICIENCY**

Autosomal recessive IKK2 deficiency (OMIM 615592) is a primary immunodeficiency disorder due to mutations in the IKBKB gene, a central component of the IKK complex in the canonical NF-kB signaling pathway (Figure 3, Table 1).8

Immunological aspects
The patients present within the first months of life with numerous bacterial, fungal and viral infections, including candidiasis,
pneumonia, bacteremia, sepsis, meningitis and osteomyelitis. Multiple and variable organisms have been isolated from these patients, including Escherichia coli, Mycobacterium avium, Listeria monocytogenes, pneumococcus, Serratia marcescens and Klebsiella. Other symptoms include chronic diarrhea and failure to thrive.43 These patients have normal B-cell and T-cell counts but very low levels of immunoglobulins, as well as a severe defect in immune-cell activation that affects both innate and adaptive immune-receptor pathways.43

Genetic aspects
Recently, mutations in the IKBKB gene were discovered to be the cause of immunodeficiencies.43 In four patients with severe combined immunodeficiency, a homozygous duplication, c.1292dupG, in the IKBKB gene resulting in a complete loss of protein function has been identified.43

Molecular aspects
IKK2-deficient patient fibroblasts show impaired phosphorylation of IκBα in response to TNF-α stimulation. Degradation of IκBα upon IL-1β stimulation is marginally affected, whereas degradation in response to toll-like receptor-5 stimulation by flagellin is absent, indicating distinct requirements for IKK2. The IL-6 response to TNF-α is normal, but it is reduced in response to LPS, and acts through TLR4. The finding of an impaired response to TNF-α, as well as to TLR4 or TLR5 stimulation indicates an additional innate immunological defect in these patients. Moreover, the NF-κB binding to DNA after TNF-α stimulation is considerably decreased in patient cells.43

**AUTOSOMAL RECESSIVE IRAK-4 DEFICIENCY**

IL-1R-associated kinase (IRAK)-4 deficiency is an autosomal recessive primary immunodeficiency (OMIM 607676) that impairs NF-κB activation in the TLR signaling pathway.8,44,45

Immunological aspects
Patients affected by IRAK-4 deficiency present recurrent infections from S. pneumoniae, S. aureus and P. aeruginosa bacteria, and are also susceptible to infections from fungi (C. albicans) and other opportunistic infections. Blood cells from these patients fail to produce IL-1β, IL-6, IL-8, IL-12, TNF-α or interferon (IFN)-γ in response to IL-1β, IL-18 or known TLR agonists, whereas their response to TNF-α is unaffected.46,47 The impact of IRAK-4 deficiency may vary from cell to cell (only blood cells and fibroblasts have been tested in IRAK-4-deficient patients). IRAK-4-deficient patients show apparently normal T- and B-cell responses, but a few patients seem to have a poor antibody response to carbohydrates, suggesting that T-independent B-cell responses might be affected.15

Genetic aspects
Autosomal recessive IRAK-4 deficiency was first discovered in 2003,48 and since then up to 49 patients have been identified.46 It is caused by homozygous or compound heterozygous mutations in the IRAK4 gene: two missense (p.Arg12Cys and p.Arg391His49); five frameshift (p.Pro42fsX450, p.Ala211fsX251, p.Asn175fsX3152, p.Thr208fsX1253 and p.Leu274fsX1454); and three nonsense (p.Tyr48X51, p.Gln293X54 and p.Glu402X55). All mutations other than the missense mutations were predicted to be loss-of-expression and loss-of-function, as they create a premature termination codon or delete a large segment of the gene.15,49–55

Molecular aspects
IRAK-4 is a member of the IRAK family of protein kinases that have an essential role in NF-κB activation in the TLR and T-cell receptor signaling pathways.44,45 IRAK-4 interacts with both MyD88 and IRAK-1, and its catalytic activity is required for IRAK-1 activation. Once hyperphosphorylated by IRAK-4, IRAK-1 associates with TNF receptor-associated factor (TRAF) 6, triggering the activation of both the NF-κB and mitogen-activated protein kinase pathways. Like other IRAKs, IRAK-4 contains two structural domains: a death domain that mediates the molecular recognition of other death domain-containing proteins, and a catalytic kinase domain.50 Moreover, in cells derived from patients, both the NF-κB and p38 activating signaling pathways were defective, suggesting that the immunodeficiency caused by IRAK-4 mutations additionally involves a perturbed mitogen-activated protein kinase signaling.57

**AUTOSOMAL RECESSIVE MYD88 DEFICIENCY**

Autosomal recessive MyD88 deficiency (OMIM 612260) is a rare primary immunodeficiency due to the Myeloid Differentiation primary response 88 (MyD88) gene, involved in the NF-κB canonical pathway in TLRs and IL-1Rs.8

Immunological aspects
Patients suffer from recurrent pyogenic bacterial infections, including invasive pneumococcal disease. The immunological phenotype of patients reported with this MyD88 deficiency is similar to that of Myd88-deficient mice, but the infectious phenotype is different. Indeed, MyD88-deficient patients are susceptible to S. aureus, P. aeruginosa and S. pneumoniae, but are normally resistant to most other infectious agents. In contrast, Myd88-deficient mice have been shown to be susceptible to most common bacteria, viruses, fungi and parasites.58

Genetic aspects
Autosomal recessive MyD88 deficiency was first discovered in 2008 and up to 24 cases have been reported.58–60 New mutations in the MyD88 gene have been reported: a homozygous in-frame MyD88 deletion (p.E52del), compound heterozygous missense mutations (p.L93P; p.R196C) and a homozygous missense mutation (p.R196C) have been identified.58 The deletion and missense mutations affected conserved residues.

Molecular aspects
MyD88 is a key downstream adapter for most TLRs and IL-1Rs that are essential for protective immunity to a small number of pyogenic bacteria. Functional analysis using patient fibroblasts and the expression of wild-type or mutant alleles in cell lines has demonstrated that p.E52del, p.R196C and p.L93P mutations result in a loss-of-function and lead to complete MyD88 deficiency.58 The MyD88 protein has been detected in SV40-transformed fibroblasts in different amounts: in trace amounts for patients with a p.E52del/p.E52del mutation, small amounts for patients with L93P/R196C and normal amounts for patients with p.R196C/p.R196C, suggesting that patients have a functional MyD88 deficiency, with low or normal MyD88 protein levels.58

**NEW IMMUNODEFICIENCY IMPAIRING THE NF-κB SIGNALING PATHWAY**

Recently, mutations in new genes were associated with primary immunodeficiency with functional defects in the canonical and non-canonical NF-κB signaling pathway.8

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Autosomal recessive and autosomal dominant TRIF deficiency (OMIM 614850)

Three unrelated cases of autosomal recessive and autosomal dominant TRIF deficiency affected by *herpes simplex encephalitis* were reported. The autosomal recessive form of the disease has been found to be due to a homozygous nonsense mutation (p.R141X) that results in a complete absence of the Toll/IL-1R domain-containing adapter inducing IFN-β (TRIF) protein. Both the TLR3- and the TRIF-dependent TLR4 signaling pathways are abolished. The autosomal dominant form of disease has been found to be due to two heterozygous missense mutations (p.P625L and p.S186L) resulting in a dysfunctional protein. In this form of the disease, the TLR3 signaling pathway is impaired, whereas the TRIF-dependent TLR4 pathway is unaffected.

Autosomal dominant TRAF3 deficiency (OMIM 614849)

Another immunodeficiency associated with a clinical phenotype of *herpes simplex encephalitis* is the autosomal dominant TRAF3 deficiency. TNF receptor-associated factor 3 (TRAF3) functions downstream of multiple receptors that induce IFN-α, IFN-β and IFN-λ production, including TLR3. The missense TRAF3 mutation (p.R118W) that proves to be responsible for the autosomal dominant predisposition to *herpes simplex encephalitis* has been reported. Previous studies have identified the p.R118W mutation of TRAF3 as a somatic mutation involved in multiple myeloma.

Autosomal recessive HOIL1 deficiency (OMIM 610924)

A new fatal inherited disorder characterized by chronic autoinflammation, invasive bacterial infections and muscular amylopectinosis has been identified: autosomal recessive HOIL1 deficiency. Patients from two kindreds carry biallelic loss-of-expression and loss-of-function mutations in *Heme-Oxidized Irp2 Ubiquitin Ligase 1* (HOIL1 also called RBCK1), a component of the linear ubiquitination chain assembly complex (LUBAC): p.Q185X and p.L415fsX7. These mutations produce an impairment of the stability of the LUBAC complex resulting in an impaired NF-κB-driven gene transcription and cytokine production in response to TNF and IL-1β. In particular, NF-κB activation in response to IL-1β is compromised in the patients’ fibroblasts. In contrast, the patients’ mononuclear leukocytes, particularly monocytes, are hyper-responsive to IL-1β. The consequences of HOIL-1 and LUBAC deficiencies for IL-1β responses thus differ between cell types, consistent with the unique association of autoinflammation and immunodeficiency in these patients.

Autosomal dominant NFKB2 deficiency (OMIM 615577)

Recently, a heterozygous 1-bp deletion (c.2564delA) in the NFKB2 gene, resulting in a frameshift and premature termination (p.K855fsX7), was identified. The mutation caused a truncation in the C-terminus of the protein, removing the conserved phosphorylation sites required for activation of p100 to p52. Another heterozygous mutation, c.2557C>T transition, in the NFKB2 gene, resulting in an p.R853X nonsense mutation, was described. This patient was identified from a cohort of 33 individuals with common variable immune deficiency (CVID) who were tested for variants in the NFKB2 gene. The mutation caused a truncation in the C-terminus of the protein, removing the conserved phosphorylation sites required for activation of p100 to p52. Liu et al. (2014) identified a heterozygous 8-bp deletion (c.2593_2600del) resulting in a frameshift and premature termination (p.D865FsX17). The protein expressed from the mutant allele was unable to be phosphorylated at regulatory residue 866, which abolished the proper processing and activation of the NF-κB signaling pathway with a consequent defect in B-cell differentiation and T follicular helper cell development.

CONCLUSION

Defects in the NF-κB activation pathway have been linked to several human diseases, including primary immunodeficiencies. NF-κB dimers are involved in the development and function of the immune system, with their activation affecting various immunity- and inflammation-associated genes such as acute-phase reactants, cytokines, chemokines, growth factors and receptors, adhesion molecules, and regulators of apoptosis and cellular proliferation. The rapid advances in gene identification technology, such as whole-genome sequencing and the examinations through integrated diagnostics of affected individuals, help clarify the infectious phenotypes associated with these genetic defects, initiating the forward genetic dissection of NF-κB-mediated immunity. In fact, the effect of several mutations in different components of the NF-κB signaling pathway demonstrates the crucial role of this pathway in human immunity to infection (Table 1). The clinical features of the MPIDs vary in severity based on the residual function of the mutated protein.

Further *in vitro* characterization of the NF-κB signaling pathways in MPID patients should improve the definition of candidate genes in other patients with unexplained infectious diseases.

ABBREVIATIONS

AD-EDA-ID, autosomal dominant ectodermal dysplasia with immunodeficiency; IκB, inhibitor of NF-κB proteins; HOIL, heme-oxidized irp2 ubiquitin ligase; IKK, IκB kinase; IRAK, IL-1R-associated kinase; LUBAC, linear ubiquitination chain assembly complex; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; TLR, Toll-Like Receptor; TNF, tumor necrosis factor; TRAF, TNF receptor-associated factor; XL-EDA-ID, X-linked anhidrotic ectodermal dysplasia with immunodeficiency

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the Incontinentia Pigmenti International Foundation (IPIF, [http://www.ipif.org/]), the association France Incontinentia Pigmenti (FIP, [http://incontinentia-pigmenti.fr/]), the Italian Incontinentia Pigmenti ASSociation (IPASSI, [http://www.incontinentiapigmenti.it/]), DHTECH, Progetto Formazione PON n°01-02342 for the fellowship to MIC and the Basilicata Innovazione [http://www.basilicatanovazione.zee.it] for supporting MP.

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