



ELSEVIER

Inherited disorders of NF- κ B-mediated immunity in man

Anne Puel¹, Capucine Picard^{1,2}, Cheng-Lung Ku¹, Asma Smahi³ and Jean-Laurent Casanova^{1,2,4}

The transcription factors of the NF- κ B family play an important role in immunity to infection in animal models. Three human primary immunodeficiencies associated with impaired NF- κ B signaling were recently described. X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) is caused by hypomorphic mutations in the gene encoding NEMO/IKK γ , the regulatory subunit of the I κ B-kinase (IKK) complex. Autosomal dominant EDA-ID (AD-EDA-ID) is caused by a hypermorphic mutation in the gene encoding the inhibitory protein I κ B α . Autosomal recessive immunodeficiency without EDA is caused by mutations in the gene encoding IRAK-4, a kinase acting upstream from the IKK complex in the TIR signaling pathway. The description of the infectious phenotypes associated with these genetic defects has initiated the forward genetic dissection of NF- κ B-mediated immunity in man.

Addresses

¹Laboratoire de Génétique Humaine des Maladies Infectieuses, Université de Paris René Descartes, Institut National de la Santé et de la Recherche Médicale U550, Faculté de Médecine Necker, 156 rue de Vaugirard, Paris 75015, France, EU

²Unité d'Immunologie et d'Hématologie Pédiatriques, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France, EU

³Unité de Recherches sur les Handicaps Génétiques de l'Enfant, Institut National de la Santé et de la Recherche Médicale U393, Hôpital Necker-Enfants Malades, 75015 Paris, France, EU

⁴e-mail: casanova@necker.fr

Current Opinion in Immunology 2004, 16:34–41

This review comes from a themed issue on Innate immunity Edited by Bruce Beutler and Jules Hoffmann

0952-7915/\$ – see front matter

© 2003 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.coi.2003.11.013

Abbreviations

AD-EDA-ID	autosomal dominant EDA-ID
EDA	anhidrotic ectodermal dysplasia
EDA-ID	EDA with immunodeficiency
IκB	inhibitors of NF- κ B
IKK	I κ B kinase
IL	interleukin
IP	incontinentia pigmenti
LCMV	lymphocytic choriomeningitis virus
IP	incontinentia pigmenti
IRAK	IL-1-receptor-associated kinase
LPS	lipopolysaccharide
NEMO	NF- κ B essential modulator
NF-κB	nuclear factor kappa B
NK	natural killer
OL-EDA-ID	osteopetrosis and lymphoedema with EDA-ID
TIR	Toll/IL-1 receptor
TNF-α	tumor necrosis factor α

TLRs Toll-like receptors
XL-EDA-ID X-linked EDA-ID

Introduction

The mammalian NF- κ B family of transcription factors is composed of homo- and hetero-dimers of five structurally related and evolutionary conserved proteins (NF- κ B1/p50, NF- κ B2/p52, RelA [also called p65], RelB and c-Rel) [1]. These transcription factors are sequestered in the cytoplasm of resting cells through associations with the inhibitor of NF- κ B (I κ B) proteins, which belong to a family that includes I κ B α , I κ B β , I κ B ϵ and the precursors of p50 and p52 (namely p105 and p100). Upon cell stimulation, I κ Bs are phosphorylated at two conserved critical amino-terminal serine residues by the I κ B kinase (IKK) complex, leading to their ubiquitination and subsequent degradation. The IKK complex is composed of at least two related catalytic subunits, IKK α /IKK1 and IKK β /IKK2, and an essential regulatory subunit IKK γ /NEMO (for NF- κ B essential modulator). The degradation of I κ Bs results in the translocation of NF- κ B dimers to the nucleus, where they bind to DNA at cognate binding sites to regulate gene transcription.

NF- κ B dimers are involved in the development and function of the immune system [2]. NF- κ B dimers are rapidly induced upon stimulation of a wide range of receptors involved in innate and/or adaptive responses, such as surface Toll-like receptors (TLRs; [3]), intracytoplasmic nucleotide oligomerization domain/caspase recruitment domain (NOD/CARD) receptors [4], members of the IL-1 receptor [5,6] and TNF receptor [7] superfamilies, the IL-15 [8] and IL-17 [9] receptors, and T- and B-cell antigen receptors [10]. NF- κ B activation leads in turn to the expression of various genes involved in immunity, such as acute-phase proteins, cytokines, chemokines, growth factors, their receptors, adhesion and co-stimulatory surface molecules, and regulators of cell proliferation and apoptosis. The various NF- κ B dimers may positively or negatively regulate their target genes.

Genetic studies performed in animal models demonstrated the critical role of NF- κ B in host defenses against pathogens. Studies in *Drosophila* have highlighted the major role played by Rel/NF- κ B orthologs in defenses against bacteria and fungi [11,12]. Several IKK-, I κ B- and NF- κ B-knockout mice die in the embryonic or perinatal periods (IKK α ^{-/-}, IKK β ^{-/-}, IKK γ /NEMO^{-/-}, I κ B α ^{-/-}, p65/RelA^{-/-}), precluding studies of their role in host defenses. However, mice that are either deficient

(p105^{-/-}, p50^{-/-}, p52^{-/-}, RelB^{-/-}, c-Rel^{-/-}) or transgenic (overexpressing a degradation-deficient I κ B α : Tg Δ N-I κ B α) for these factors are viable [13,14]. Some of these mice have normal or increased resistance, compared to wild-type mice, to infection by various microorganisms, such as bacteria: *Listeria monocytogenes*, *Shigella flexneri* (I κ B ϵ ^{-/-}; [15]), *Haemophilus influenzae*, *Escherichia coli* (p50^{-/-}; [16]); parasites: *Trichus muris*, *Toxoplasma gondii* (c-rel^{-/-}; [17,18]); and viruses: lymphocytic choriomeningitis virus (LCMV; c-Rel^{-/-}; [19]) or murine encephalomyocarditis (p50^{-/-}; [16]).

The same or other mice, however, were also shown to be susceptible to various infections, such as bacteria: *L. monocytogenes*, *Streptococcus pneumoniae* (p50^{-/-}, p52^{-/-}, RelB^{-/-}, c-Rel^{-/-}, Tg Δ N-I κ B α ; [16,19–22]), *Helicobacter hepaticus* (p50^{-/-}; [23,24]), *Staphylococcus xylois* and *Pasteurella haemolytica* (p105^{-/-}, c-Rel^{-/-}; [19,25]); parasites: *Leishmania major* (p50^{-/-}, p52^{-/-}, c-Rel^{-/-}; [26–28]), *T. muris* (p50^{-/-}, p52^{-/-}; [17]), *T. gondii* (p52^{-/-}, RelB^{-/-}, Tg Δ N-I κ B α ; [29–31]); and viruses: LCMV (RelB^{-/-}; [22]) and influenza virus (c-Rel^{-/-}; [32]). These studies have initiated a genetic dissection of the role of the different molecules involved in NF- κ B activation in achieving protective immunity to infection in the mouse model.

In this review we will describe three recently identified human primary immunodeficiencies involving the NF- κ B signaling pathway, with a particular emphasis on the associated infectious diseases. We will not discuss other inherited NF- κ B disorders that are not associated with an immunodeficiency, such as incontinentia pigmenti (IP; lethal *in utero* for males, resulting from amorphic *NEMO* mutations) or anhidrotic ectodermal dysplasia (a purely developmental defect, resulting from amorphic mutations in *EDA/ED1*, *EDAR*, *EDARADD* genes, encoding ectodysplasin, its receptor, and the EDAR-associated death domain proteins, respectively; [33]).

X-linked anhidrotic ectodermal dysplasia with immunodeficiency

Patients with anhidrotic ectodermal dysplasia (EDA) display abnormal development of ectoderm-derived structures, with hypo- or ano-dontia (partial or total absence of teeth, respectively), conical teeth, dry skin (due to the absence or rarity of eccrine sweat glands), and hypohidrosis with sparse scalp hair and eyebrows [34]. Patients with EDA and immunodeficiency (EDA-ID) present some or all of these features, together with severe infectious diseases [35]. The first known clinical report of EDA-ID was published in 1986 and concerned a 20-month-old boy with EDA who died from acute miliary tuberculosis [36]. Immunological abnormalities associated with EDA were first described in 1995 by Abinun [37]. Thirty-one patients with EDA-ID have been described [35,36,38–49]. EDA-ID principally affects boys, suggesting X-linked recessive inheritance (XL-

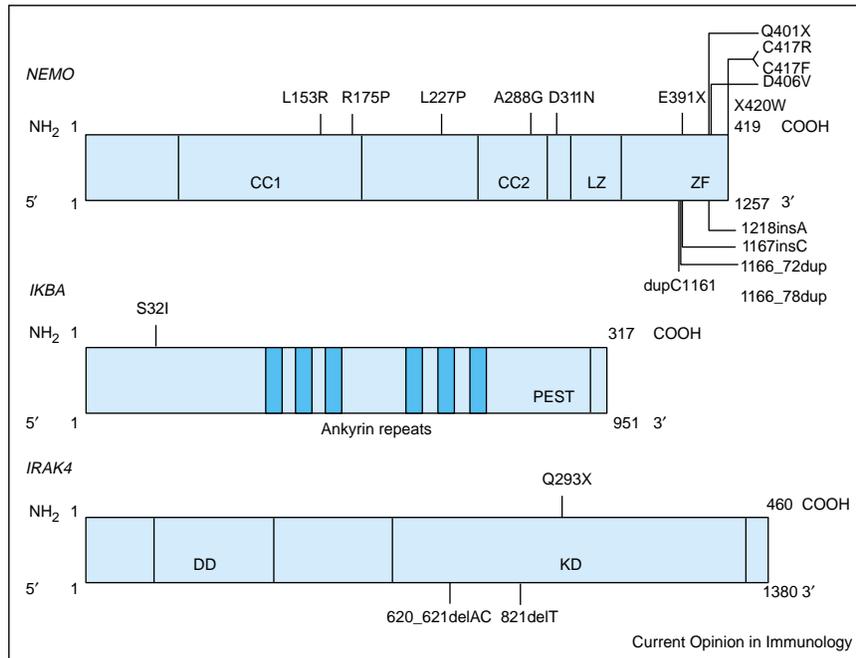
EDA-ID). This was confirmed in 2000 and beyond with the identification of disease-causing hypomorphic mutations in *NEMO*, which is located on the X chromosome [35,41,43–49].

XL-EDA-ID patients generally suffer in early childhood from multiple and severe bacterial infections of the respiratory and gastrointestinal tracts, skin, soft tissues and bones, together with meningitis and septicemia [35–49]. These infections are mostly caused by Gram-positive (*Streptococcus pneumoniae*, *Staphylococcus aureus*) and Gram-negative (*H. influenzae*) encapsulated pyogenic bacteria. Several patients also suffered from severe mycobacterial disease (*Mycobacterium avium*), and there have been rare cases of children suffering from fungal (*Pneumocystis carinii*; [45,48]) and viral (*Cytomegalovirus*; [38,49]) diseases. Many patients show poor clinical and biological inflammatory responses during infection episodes. The nature and severity of infections vary from case to case, and 15 out of the 31 patients described have died of infections.

All patients tested to date have presented a lack of polysaccharide-specific antibodies, despite infection by, and/or vaccination against, encapsulated bacteria [35,39,40,43,45, 47–49]. This may partly account for the susceptibility of these patients to infections with pyogenic bacteria [50,51]. Several patients present low serum IgG (especially IgG₂) levels, and/or high serum IgM levels [40,43,45–48], whereas the levels of IgA vary (hyper IgM-like phenotype). Three patients with impaired natural killer (NK)-cell cytotoxic activity, despite normal numbers of peripheral blood NK cells, have been reported [49]. Other diagnostic immunological parameters, such as the number of blood phagocytes, NK cells, and B and T lymphocytes, lymphocyte subset distribution (CD4⁺, CD8⁺), T-cell proliferation in response to mitogens and antigens, and antibody responses to protein antigens, were generally normal in all patients tested. The severity of the infectious phenotype of XL-EDA-ID thus contrasts with the paucity of detectable abnormalities in routine immunological examination.

Following the identification, by positional cloning, of amorphic *NEMO* mutations as the cause of IP [41], which is usually lethal *in utero* for males, hypomorphic mutations in *NEMO* have been identified in XL-EDA-ID patients (Figure 1; [35,38–41,43–49]). These *NEMO* mutations impaired, but did not abolish, NF- κ B signaling [45]. In males, the *NEMO* genotype therefore correlated with developmental and infectious phenotypes [44–46, 48,52]. Amorphic mutations cause lethal IP *in utero*, hypomorphic mutations in the coding region cause XL-EDA-ID, and hypomorphic mutation of the stop codon (X420W) causes the most severe form of EDA-ID, which is associated not only with osteopetrosis and lymphoedema (XL-OL-EDA-ID), but also with more diverse and severe infections [41,45,47,48]. EDA results from

Figure 1



Schematic representation of the coding region, domain organization and mutations of the proteins encoded by the three disease-causing genes associated with impaired NF- κ B immunity: NEMO, with the coil-coiled (CC), leucine zipper (LZ) and zinc finger (ZF) domains; I κ B α with the ankyrin repeat and PEST (proline/glutamic acid/serine/threonine) domains; IRAK-4, with the death domain (DD) and kinase domain (KD). The position of all mutations reported is represented (amino acid nomenclature on the top, nucleotide nomenclature at the bottom).

impaired NEMO-dependent NF- κ B activation in response to ectodysplasin [45,53]. Osteopetrosis and lymphoedema probably result from impaired NF- κ B activation via the receptor activator of NF- κ B (RANK; [54]), and the vascular-endothelial growth factor receptor-3 (VEGFR-3; [55]), respectively.

In contrast, the pathogenesis of abnormalities detected in the immunological profile, such as the lack of antibody responses against polysaccharides, is much less clear because of the number of receptors involved in NF- κ B-mediated immunity (Figure 2). Moreover, in one study, the patients' cells showed impaired responses to IL-1 β , IL-18, lipopolysaccharide (LPS), TNF- α and to some, but not all, CD40-ligand assays [45], whereas, in another study, the patients' cells showed impaired responses to all CD40 ligand assays tested, but not to LPS, SAC (*Staphylococcus aureus* [Cowan strain]) and TNF- α [46]. This suggests that the immunological abnormalities depend on the NEMO mutation involved. The pathogenesis of infections is even more obscure. Intact NEMO-dependent NF- κ B activation in humans is clearly important for the control of pyogenic bacteria and mycobacteria. Susceptibility to fungi and viruses in a few patients suggests that NEMO plays a broader role in immunity to infection. The identification in other patients of germline mutations in other genes, the products of which act upstream or downstream from NEMO,

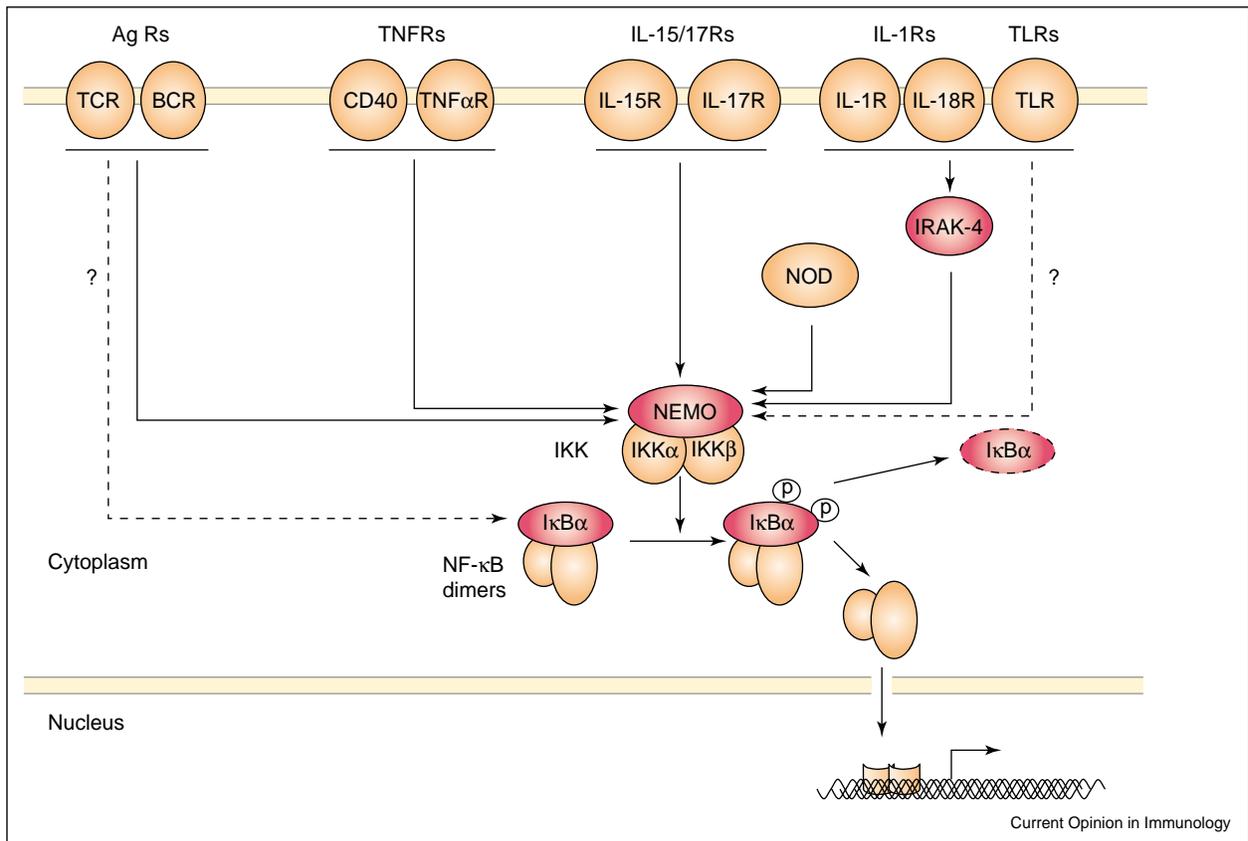
would be the most direct way to determine which immunological defects are responsible for infections in NEMO-deficient children.

Autosomal dominant EDA-ID

A novel autosomal dominant form of EDA-ID, recently identified in one child, was found to be caused by a hypermorphic mutation of the gene encoding I κ B α [56*]. Similar to XL-EDA-ID patients, from the age of two months he suffered from multiple and severe infections with several Gram-positive and Gram-negative bacteria, leading to chronic bronchopneumonitis and gastroenteritis, with failure to thrive. His immunological profile showed normal percentages of T, B and NK cells, a normal distribution of T-lymphocyte subsets (CD4⁺, CD8⁺), and normal NK-cell activity. He had high serum IgM levels, and low serum IgG and IgA levels. Unlike patients with XL-EDA-ID, however, he presented leukocytosis with lymphocytosis and no serum antibodies specific for any recall antigens, including protein antigens. His clinical course was extremely severe, with bone marrow transplantation required by the time he was eight months old.

The severity of the case was due to a unique, severe T-cell abnormality. This patient had no detectable γ/δ T cells and no memory α/β T cells, as all his CD4⁺ and CD8⁺ cells were of the naïve CD45RA phenotype. An

Figure 2



Immune receptor-signaling pathways leading to NF- κ B activation are grouped in four categories according to surface receptors: antigen receptors (TCR and BCR), members of the TNF receptor superfamily (TNF-Rs), represented by CD40 and TNF α R, IL-15/IL-17 receptors (which do not form a superfamily but are assembled by commodity), and members of the TIR superfamily (IL-1Rs/TLRs), and intracellular receptors (NOD). The three proteins of the NF- κ B signaling pathway (IRAK-4, NEMO and I κ B α) responsible for primary immunodeficiency are shown in a darker shade.

in-depth study of the T-cell phenotype showed a complete lack of proliferation *in vitro* in response to anti-CD3, with restoration of proliferation on addition of recombinant IL-2 or anti-CD28. By contrast, proliferation in response to lectins, allogeneic cells and phorbol 12-myristate 13-acetate (PMA)-ionomycin was normal. The patient's T cells also failed to respond to all recall antigens tested. However, T-cell blasts generated *in vitro* acquired the HLA-DR, CD25 and CD45RO markers and responded to anti-CD3 stimulation. By contrast, known XL-EDA-ID patients have normal numbers of memory T cells in the blood, and their T cells proliferate *in vitro* in response to anti-CD3 or antigens.

A heterozygous *IKBA* missense mutation has been identified (Figure 1) that converts the phospho-acceptor serine in position 32 of the protein to isoleucine (S32I), impairing the phosphorylation of I κ B α by IKK. After treatment with TNF- α or IL-1 β , almost no degradation of I κ B α was observed in the patient's fibroblasts, whereas the degradation of I κ B β and I κ B ϵ was normal; by contrast, in patients with XL-OL-EDA-ID, impairment is

observed in the degradation of I κ B α , I κ B β and I κ B ϵ [45,56]. Accordingly, no Ser32-phosphorylated I κ B α was detected in the patient's fibroblasts upon activation. So, as observed in patients with XL-OL-EDA-ID, the patient's fibroblasts responded poorly to TNF- α and IL-1 β to induce NF- κ B DNA binding activity and IL-6 secretion, and his blood cells showed impaired responses to ligands of the Toll/IL-1 receptor (TIR)-bearing receptor family, LPS and IL-1 β (Figure 2).

This S32I substitution is thus hypermorphic, as it prevents I κ B α degradation, thereby enhancing the NF- κ B-inhibitory capacity of this molecule and resulting in impaired NF- κ B activation. Similar mutations of serine 32 are gain-of-function and dominant, and have commonly been used to block NF- κ B activation [56]. The dominant nature of the S32I mutation has been demonstrated *in vitro* by the strong inhibition of NF- κ B activation upon stimulation by TNF- α of cells expressing both wild-type and mutant (S32I) I κ B α constructs [56]. Hypomorphic recessive mutations of *NEMO* and a hypermorphic dominant mutation of *IKBA* thus both lead to

EDA-ID, unambiguously indicating that the human NF- κ B signaling pathway is crucial for both normal ectodermal structure development and immunity to infection.

Notably, the child with the *IKBA* mutation underwent bone marrow transplantation early in life due to the clinical consequence of a severe T-cell phenotype resembling that of SCID patients (who lack T-cell immunity). Indeed, a distinct feature of AD-EDA-ID is the lack of memory T cells *in vivo*. This lack of memory T cells probably results from a deficient antigen-driven T-cell receptor (TCR) stimulation of naïve T cells, as suggested by the lack of naïve T-cell responses to recall antigens and anti-CD3, and the ability to generate stable memory T cells in response to more potent stimuli *in vitro*. The T-cell phenotype in the AD-EDA-ID patient absent in known XL-EDA-ID patients, may reflect NEMO-independent NF- κ B signaling in response to TCR/CD3-ligation. The NF- κ B-inducing kinase (NIK) was recently shown to be involved in T-cell activation, in which it sets the activation threshold [57], acting independently of NEMO [58]. Alternatively, the composition and/or number of NEMO-dependent NF- κ B dimers activated upon stimulation may differ between cells with *NEMO* and *IKBA* mutation. In either case, NF- κ B signaling is probably crucial for immunity to a large number of microorganisms. The present identification of only a single *IKBA*-mutated and 31 *NEMO*-mutated patients, with residual NF- κ B signaling, however, precludes the drawing of definitive conclusions.

Immunodeficiency without EDA, associated with IRAK-4 deficiency

IL-1-receptor-associated kinase (IRAK)-4 is a kinase that acts upstream from IKK in the Toll/IL-1 receptor (TIR) signaling pathway [59]. Three unrelated children with IRAK-4 deficiency were recently reported [60,61^{••}]. They developed multiple life-threatening infections with pyogenic bacteria, with no developmental signs such as EDA, osteopetrosis or lymphoedema. These patients were mostly infected with encapsulated Gram-positive *S. pneumoniae* and *S. aureus* bacteria (Table 1). No infections with *H. influenzae* were reported, but one patient

presented severe infections with other Gram-negative bacteria. These infections occurred early in life, but the condition of the children improved with age (they are now 7, 8 and 12 years old). A fourth patient was subsequently reported, confirming the clinical phenotype of IRAK-4 deficiency, as she was 21 years old and showed a lack of severe infections since the end of adolescence [62,63[•]]. The clinical improvement seen with age in the four patients [61^{••},63[•]] suggests that other immune mechanisms, probably involved in adaptive immunity, progressively come into effect, circumventing the IRAK-4 deficiency and protecting these patients against infections. Similar to EDA-ID patients, these patients displayed poor inflammatory responses during infectious episodes and routine immunological evaluations of T, B and NK lymphocytes were normal. Unlike XL-EDA-ID patients, however, these patients had apparently normal antibody responses to polysaccharide antigens, including specific antibodies against *S. pneumoniae* and, unlike AD-EDA-ID patients, they had normal T-cell phenotypes and responses to antigens.

Homozygous mutations in the *IRAK4* gene were reported for three of these patients. The mutations were amorphic, resulting in premature stop codons in the region of the gene encoding the kinase domain, resulting in a lack of detectable IRAK-4 protein (Figure 1; [61^{••}]). The fourth patient presented compound heterozygous mutations (Figure 1; [63[•]]). *In vitro* characterization of the defect showed an absence of IRAK-1 degradation upon stimulation with IL-1 β . Further downstream, levels of I κ B α degradation, NF- κ B dimer DNA binding, p38 mitogen-activated protein kinase (MAPK) activation, extracellular signal-related kinase (ERK)-1/ERK-2 activation, and IL-6 production were all low in fibroblasts stimulated with IL-1 β , whereas the response to TNF- α remained unaffected (Figures 2 and 3). Blood cells also showed profound defects in response to all IL-1 receptor- and TLR-ligands tested, although they responded normally to TNF- α [61^{••}]. The response to whole bacteria (*S. aureus*, *M. tuberculosis* and *E. coli*) was impaired but not abolished, indicating that TLR-independent pathways could serve to recognize pathogens.

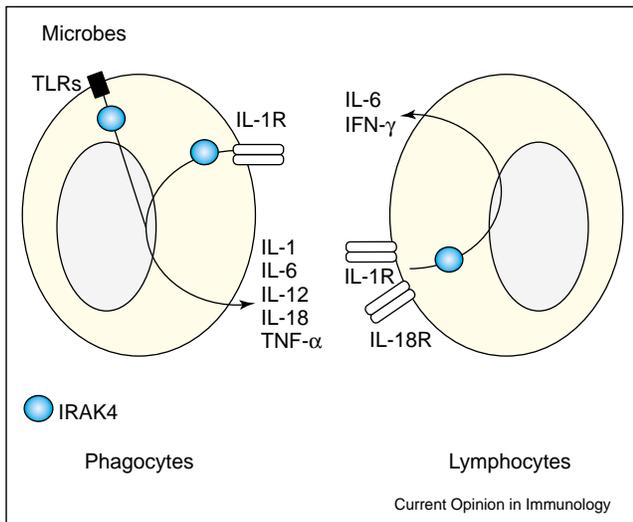
Table 1

A comparison of XL-EDA-ID, AD-EDA-ID and IRAK-4 deficiency.

Gene	Inheritance	Mutation	EDA (OL)	Infections ^a		
				Pyogenic bacteria	Mycobacteria	Other
<i>NEMO</i>	XR	Hypomorphic	+	+	+	±
<i>IKBA</i>	AD	Hypermorphic	+	+	+?	+?
<i>IRAK4</i>	AR	Amorphic	-	+	-	-

^aThe single patient with AD-EDA-ID received a heterologous hematopoietic stem cell transplantation by eight months of age, precluding a thorough description of his infectious phenotype. His profound T-cell defect, however, strongly suggests that AD-EAD-ID is not only associated with mycobacterial disease, but also with a variety of other severe infectious diseases.

Figure 3



Schematic representation of the impact of IRAK-4 deficiency on the immune and pro-inflammatory cooperation between phagocytes (polymorphonuclear neutrophils, monocytes, dendritic cells) and lymphocytes (T, B, NK cells). IRAK-4 deficiency is associated with an impaired ignition of inflammation by the ten TLR ligands, and an impaired propagation of inflammation by IL-1 and IL-18. IRAK-4 deficiency also blocks MAPK pathways (not represented). Orphan receptors of the IL-1R family are also not represented.

Unlike XL-EDA-ID, which results from hypomorphic *NEMO* mutations, or AD-EDA-ID, which results from a hypermorphic *IKBA* mutation, amorphic *IRAK4* mutations are associated with a TIR immunodeficiency without EDA. This makes it possible to define the role of the human TIR-IRAK pathway in host defenses, which is more difficult in XL- and AD-EDA-ID due to residual NF- κ B signaling and immunity. IRAK-4-deficient children suffer from severe infections caused by pyogenic encapsulated bacteria (mostly but not exclusively Gram-positive bacteria) indicating that impaired TIR signaling plays a crucial role in the development of pyogenic bacterial infections in EDA-ID. IRAK-4-deficient patients also appear to be resistant to other common microorganisms, including other bacteria, parasites, viruses and fungi. In particular, mycobacteria, such as *M. avium*, which have frequently been shown to cause disease in EDA-ID patients, or *P. carinii* and CMV more rarely involved, do not appear to be a threat to IRAK-4-deficient patients. Taking into account that IRAK-4 deficiency also blocks MAPK activation, this suggests that IRAK-4-dependent NF- κ B signaling is important for protection against pyogenic bacteria, but dispensable for immunity to most other infectious agents.

Whereas there is no mouse model bearing the *NEMO* or *IKBA* mutations found in EDA-ID patients, mice deficient for *Irak4* have been challenged with LCMV and *S.*

aureus; they were found to have impaired responses and be highly susceptible, respectively [64]. Mice deficient for other components of the TIR-signaling pathway [61**] are susceptible not only to pyogenic bacteria (notably *S. aureus* and *S. pneumoniae*), but also to a broader spectrum of infections than IRAK4-deficient patients [61**]. The difference between mice and humans may result from the protection conferred by TIR-independent signaling pathways in humans. It is also possible that IRAK-4-independent TIR signaling pathways operate in humans and mice [65,66]. It will be particularly important to test the induction of IFN- α/β in IRAK-4-deficient patients. Alternatively, the difference may be due to the fact that human infections, unlike infections in animal models, occur in natural as opposed to experimental conditions [67,68]. In any event, although the description of more patients is required before we can build up a definitive picture of the clinical phenotype of IRAK-4 deficiency, vulnerability to pyogenic bacteria will probably remain the dominant phenotype.

Conclusions

NEMO, *IKBA* and *IRAK4* mutations result in novel primary immunodeficiencies affecting the NF- κ B signaling pathway, thereby demonstrating the crucial role of this pathway in human immunity to infection. The infectious phenotypes associated with each of the three genetic disorders, however, are markedly different. XL-EDA-ID is associated with susceptibility to various bacteria, including mycobacteria, and occasionally other microbes such as fungi and viruses. A wide range of infectious phenotypes is observed in patients with XL-EDA-ID, perhaps reflecting the diversity of *NEMO* genotypes. AD-EDA-ID was identified in only one patient and is associated with multiple bacterial infections. The very profound T-cell deficiency in this child, however, supports the hypothesis that AD-EDA-ID is potentially associated with a much broader susceptibility to infection. By contrast, IRAK-4-deficient patients are susceptible to pyogenic bacteria and resistant to most other common microorganisms, including mycobacteria, viruses and fungi.

The roles in host defense of each NF- κ B subunit, of each surface receptor upstream from NF- κ B, of the signaling molecules acting in between them, and of target genes downstream from NF- κ B remain to be determined. Comprehensive *in vitro* characterization of the NF- κ B signaling pathways in patients with *NEMO*, *IKBA* or *IRAK4* mutations should improve the definition of candidate genes in other patients with unexplained infectious diseases. Novel disease-causing mutated genes will be searched for, particularly in patients with bacterial infections and EDA polysaccharide-specific antibody deficiency and/or low levels of inflammation. These bacterial infectious diseases will form the phenotypic basis for the forward genetic dissection of NF- κ B-mediated immunity to infection. The genetic dissection

of NF- κ B immunity in humans will provide complementary information to the animal models, as immunity to infection occurs in natural, as opposed to experimental, conditions in humans [67,68]. It should also be of considerable benefit to the affected patients and their families.

Acknowledgements

We thank Laurent Abel, Mario Abinun, Marion Bonnet, Jacinta Bustamante, Gilles Courtois, Orchidée Filipe Santos, Alain Israel, Françoise Le Deist, Arnold Munnich, Janine Reichenbach and members of the Laboratory of Human Genetics of Infectious Diseases for helpful discussions.

References and recommended reading

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

- of special interest
 - of outstanding interest
1. Karin M, Ben-Neriah Y: **Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity.** *Annu Rev Immunol* 2000, **18**:621-663.
 2. Li Q, Verma IM: **NF-kappaB regulation in the immune system.** *Nat Rev Immunol* 2002, **2**:725-734.
 3. Takeda K, Kaisho T, Akira S: **Toll-like receptors.** *Annu Rev Immunol* 2003, **21**:335-376.
 4. Inohara N, Nunez G: **NODs: intracellular proteins involved in inflammation and apoptosis.** *Nat Rev Immunol* 2003, **3**:371-382.
 5. Dinarello CA: **Interleukin-1.** *Cytokine Growth Factor Rev* 1997, **8**:253-265.
 6. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: **Interleukin-18 regulates both Th1 and Th2 responses.** *Annu Rev Immunol* 2001, **19**:423-474.
 7. Aggarwal BB: **Signaling pathways of the TNF superfamily: a double-edged sword.** *Nat Rev Immunol* 2003, **3**:745-756.
 8. Musikacharoen T, Matsuguchi T, Kikuchi T, Yoshikai Y: **NF-kappa B and STAT5 play important roles in the regulation of mouse Toll-like receptor 2 gene expression.** *J Immunol* 2001, **166**:4516-4524.
 9. Schwandner R, Yamaguchi K, Cao Z: **Requirement of tumor necrosis factor receptor-associated factor (TRAF)6 in interleukin 17 signal transduction.** *J Exp Med* 2000, **191**:1233-1240.
 10. Ruland J, Mak TW: **Transducing signals from antigen receptors to nuclear factor kappaB.** *Immunol Rev* 2003, **193**:93-100.
 11. Tzou P, De Gregorio E, Lemaitre B: **How *Drosophila* combats microbial infection: a model to study innate immunity and host-pathogen interactions.** *Curr Opin Microbiol* 2002, **5**:102-110.
 12. Hoffmann JA: **The immune response of *Drosophila*.** *Nature* 2003, **426**:33-38.
 13. Gerondakis S, Grossmann M, Nakamura Y, Pohl T, Grumont R: **Genetic approaches in mice to understand Rel/NF-kappaB and IkappaB function: transgenics and knockouts.** *Oncogene* 1999, **18**:6888-6895.
 14. Caamano J, Hunter CA: **NF-kappaB family of transcription factors: central regulators of innate and adaptive immune functions.** *Clin Microbiol Rev* 2002, **15**:414-429.
 15. Memet S, Laouini D, Epinat JC, Whiteside ST, Goudeau B, Philpott D, Kayal S, Sansonetti PJ, Berche P, Kanellopoulos J *et al.*: **I κ B ϵ -deficient mice: reduction of one T cell precursor subspecies and enhanced Ig isotype switching and cytokine synthesis.** *J Immunol* 1999, **163**:5994-6005.
 16. Sha WC, Liou HC, Tuomanen EI, Baltimore D: **Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses.** *Cell* 1995, **80**:321-330.
 17. Artis D, Shapira S, Mason N, Speirs KM, Goldschmidt M, Caamano J, Liou HC, Hunter CA, Scott P: **Differential requirement for NF-kappa B family members in control of helminth infection and intestinal inflammation.** *J Immunol* 2002, **169**:4481-4487.
 18. Mason N, Aliberti J, Caamano JC, Liou HC, Hunter CA: **Cutting edge: identification of c-Rel-dependent and -independent pathways of IL-12 production during infectious and inflammatory stimuli.** *J Immunol* 2002, **168**:2590-2594.
 19. Carrasco D, Cheng J, Lewin A, Warr G, Yang H, Rizzo C, Rosas F, Snapper C, Bravo R: **Multiple hemopoietic defects and lymphoid hyperplasia in mice lacking the transcriptional activation domain of the c-Rel protein.** *J Exp Med* 1998, **187**:973-984.
 20. Franzoso G, Carlson L, Poljak L, Shores EW, Epstein S, Leonardi A, Grinberg A, Tran T, Scharon-Kersten T, Anver M *et al.*: **Mice deficient in nuclear factor (NF)-kappa B/p52 present with defects in humoral responses, germinal center reactions, and splenic microarchitecture.** *J Exp Med* 1998, **187**:147-159.
 21. Lavon I, Goldberg I, Amit S, Landsman L, Jung S, Tsuberi BZ, Barshack I, Kopolovic J, Galun E, Bujard H *et al.*: **High susceptibility to bacterial infection, but no liver dysfunction, in mice compromised for hepatocyte NF-kappaB activation.** *Nat Med* 2000, **6**:573-577.
 22. Weih F, Warr G, Yang H, Bravo R: **Multifocal defects in immune responses in RelB-deficient mice.** *J Immunol* 1997, **158**:5211-5218.
 23. Erdman S, Fox JG, Dangler CA, Feldman D, Horwitz BH: **Typhlocolitis in NF-kappa B-deficient mice.** *J Immunol* 2001, **166**:1443-1447.
 24. Tomczak MF, Erdman SE, Poutahidis T, Rogers AB, Holcombe H, Plank B, Fox JG, Horwitz BH: **NF-kappaB is required within the innate immune system to inhibit microflora-induced colitis and expression of IL-12 p40.** *J Immunol* 2003, **171**:1484-1492.
 25. Ishikawa H, Claudio E, Dambach D, Raventos-Suarez C, Ryan C, Bravo R: **Chronic inflammation and susceptibility to bacterial infections in mice lacking the polypeptide (p)105 precursor (NF-kappaB1) but expressing p50.** *J Exp Med* 1998, **187**:985-996.
 26. Artis D, Speirs K, Joyce K, Goldschmidt M, Caamano J, Hunter CA, Scott P: **NF-kappa B1 is required for optimal CD4+ Th1 cell development and resistance to *Leishmania major*.** *J Immunol* 2003, **170**:1995-2003.
 27. Grigoriadis G, Zhan Y, Grumont RJ, Metcalf D, Handman E, Cheers C, Gerondakis S: **The Rel subunit of NF-kappaB-like transcription factors is a positive and negative regulator of macrophage gene expression: distinct roles for Rel in different macrophage populations.** *EMBO J* 1996, **15**:7099-7107.
 28. Speirs K, Caamano J, Goldschmidt MH, Hunter CA, Scott P: **NF-kappa B2 is required for optimal CD40-induced IL-12 production but dispensable for Th1 cell Differentiation.** *J Immunol* 2002, **168**:4406-4413.
 29. Caamano J, Tato C, Cai G, Villegas EN, Speirs K, Craig L, Alexander J, Hunter CA: **Identification of a role for NF-kappa B2 in the regulation of apoptosis and in maintenance of T cell-mediated immunity to *Toxoplasma gondii*.** *J Immunol* 2000, **165**:5720-5728.
 30. Caamano J, Alexander J, Craig L, Bravo R, Hunter CA: **The NF-kappa B family member RelB is required for innate and adaptive immunity to *Toxoplasma gondii*.** *J Immunol* 1999, **163**:4453-4461.
 31. Tato CM, Villarino A, Caamano JH, Boothby M, Hunter CA: **Inhibition of NF-kappa B activity in T and NK cells results in defective effector cell expansion and production of IFN-gamma required for resistance to *Toxoplasma gondii*.** *J Immunol* 2003, **170**:3139-3146.
 32. Harling-McNabb L, Deliyannis G, Jackson DC, Gerondakis S, Grigoriadis G, Brown LE: **Mice lacking the transcription factor subunit Rel can clear an influenza infection and have functional anti-viral cytotoxic T cells but do not develop an optimal antibody response.** *Int Immunol* 1999, **11**:1431-1439.
 33. Orange JS, Geha RS: **Finding NEMO: genetic disorders of NF-[kappa]B activation.** *J Clin Invest* 2003, **112**:983-985.

34. Clarke A, Phillips DI, Brown R, Harper PS: **Clinical aspects of X-linked hypohidrotic ectodermal dysplasia**. *Arch Dis Child* 1987, **62**:989-996.
35. Carrol ED, Gennery AR, Flood TJ, Spickett GP, Abinun M: **Anhidrotic ectodermal dysplasia and immunodeficiency: the role of NEMO**. *Arch Dis Child* 2003, **88**:340-341.
36. Frix CD 3rd, Bronson DM: **Acute miliary tuberculosis in a child with anhidrotic ectodermal dysplasia**. *Pediatr Dermatol* 1986, **3**:464-467.
37. Abinun M: **Ectodermal dysplasia and immunodeficiency**. *Arch Dis Child* 1995, **73**:185.
38. Sitton JE, Reimund EL: **Extramedullary hematopoiesis of the cranial dura and anhidrotic ectodermal dysplasia**. *Neuropediatrics* 1992, **23**:108-110.
39. Abinun M, Spickett G, Appleton AL, Flood T, Cant AJ: **Anhidrotic ectodermal dysplasia associated with specific antibody deficiency**. *Eur J Pediatr* 1996, **155**:146-147.
40. Schweizer P, Kalthoff H, Horneff G, Wahn V, Diekmann L: **[Polysaccharide specific humoral immunodeficiency in ectodermal dysplasia. Case report of a boy with two affected brothers]**. *Klin Padiatr* 1999, **211**:459-461.
41. Smahi A, Courtois G, Vabres P, Yamaoka S, Heuertz S, Munnich A, Israel A, Heiss NS, Klauck SM, Kioschis 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**:466-472.
42. Smythe WR, Bridges ND, Gaynor JW, Nicolson S, Clark BJ, Spray TL: **Bilateral sequential lung transplant for ectodermal dysplasia**. *Ann Thorac Surg* 2000, **70**:654-656.
43. Zonana J, Elder ME, Schneider LC, Orlov SJ, Moss C, Golabi M, Shapira SK, Farndon PA, Wara DW, Emmal SA 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**:1555-1562.
44. Aradhya S, Courtois G, Rajkovic A, Lewis RA, Levy M, Israel A, Nelson DL: **Atypical forms of incontinentia pigmenti in male individuals result from mutations of a cytosine tract in exon 10 of NEMO (IKK-gamma)**. *Am J Hum Genet* 2001, **68**:765-771.
45. Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, Bodemer C, Kenwick S, Dupuis-Girod S, Blanche S et al.: **X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling**. *Nat Genet* 2001, **27**:277-285.
46. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W: **Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohidrotic ectodermal dysplasia**. *Nat Immunol* 2001, **2**:223-228.
47. Mansour S, Woffendin H, Mitton S, Jeffery I, Jakins T, Kenwick S, Murday VA: **Incontinentia pigmenti in a surviving male is accompanied by hypohidrotic ectodermal dysplasia and recurrent infection**. *Am J Med Genet* 2001, **99**:172-177.
48. Dupuis-Girod S, Corradini N, Hadj-Rabia S, Fournet JC, Faivre L, Le Deist F, Durand P, Doffinger R, Smahi A, Israel A et al.: **Osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency in a boy and incontinentia pigmenti in his mother**. *Pediatrics* 2002, **109**:e97.
49. Orange JS, Brodeur SR, Jain A, Bonilla FA, Schneider LC, Kretschmer R, Nurko S, Rasmussen WL, Kohler JR, Gellis SE et al.: **Deficient natural killer cell cytotoxicity in patients with IKK-gamma/NEMO mutations**. *J Clin Invest* 2002, **109**:1501-1509.
50. Sanders LA, Rijkers GT, Kuis W, Tenbergen-Meekes AJ, de Graeff-Meeder BR, Hiemstra I, Zegers BJ: **Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections**. *J Allergy Clin Immunol* 1993, **91**:110-119.
51. Rijkers GT, Sanders LA, Zegers BJ: **Anti-capsular polysaccharide antibody deficiency states**. *Immunodeficiency* 1993, **5**:1-21.
52. Makris C, Roberts JL, Karin M: **The carboxyl-terminal region of IkkappaB kinase gamma (IKKgamma) is required for full IKK activation**. *Mol Cell Biol* 2002, **22**:6573-6581.
53. Smahi A, Courtois G, Rabia SH, Doffinger R, Bodemer C, Munnich A, Casanova JL, Israel A: **The NF-kappaB signaling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes**. *Hum Mol Genet* 2002, **11**:2371-2375.
54. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A et al.: **OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis**. *Nature* 1999, **397**:315-323.
55. Karkkainen MJ, Ferrell RE, Lawrence EC, Kimak MA, Levinson KL, McTigue MA, Alitalo K, Finegold DN: **Missense mutations interfere with VEGFR-3 signaling in primary lymphoedema**. *Nat Genet* 2000, **25**:153-159.
56. Courtois G, Smahi A, Reichenbach J, Doffinger R, Cancrini C, Bonnet M, Puel A, Chable-Bessia C, Yamaoka S, Feinberg J et al.: **A hypermorphic IkkB α mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency**. *J Clin Invest* 2003, **112**.
- The first description of an autosomal dominant form of anhidrotic ectodermal dysplasia associated with a unique and severe T-cell immunodeficiency.
57. Matsumoto M, Yamada T, Yoshinaga SK, Boone T, Horan T, Fujita S, Li Y, Mitani T: **Essential role of NF-kappa B-inducing kinase in T cell activation through the TCR/CD3 pathway**. *J Immunol* 2002, **169**:1151-1158.
58. Pomerantz JL, Baltimore D: **Two pathways to NF-kappaB**. *Mol Cell* 2002, **10**:693-695.
59. Janssens S, Beyaert R: **Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members**. *Mol Cell* 2003, **11**:293-302.
60. Haraguchi S, Day NK, Nelson RP Jr, Emmanuel P, Duplantier JE, Christodoulou CS, Good RA: **Interleukin 12 deficiency associated with recurrent infections**. *Proc Natl Acad Sci USA* 1998, **95**:13125-13129.
61. Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, Soudais C, Dupuis S, Feinberg J, Fieschi C et al.: **Pyogenic bacterial infections in humans with IRAK-4 deficiency**. *Science* 2003, **299**:2076-2079.
- The first description of inherited IRAK-4 deficiency in man.
62. Kuhns DB, Long Priel DA, Gallin JI: **Endotoxin and IL-1 hyporesponsiveness in a patient with recurrent bacterial infections**. *J Immunol* 1997, **158**:3959-3964.
63. Medvedev AE, Lentschat A, Kuhns DB, Blanco JC, Salkowski C, Zhang S, Arditi M, Gallin JI, Vogel SN: **Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections**. *J Exp Med* 2003, **198**:521-531.
- The description of a 21-year-old compound heterozygous IRAK-4-deficient patient.
64. Suzuki N, Suzuki S, Duncan GS, Millar DG, Wada T, Mirtsos C, Takada H, Wakeham A, Itie A, Li S et al.: **Severe impairment of interleukin-1 and Toll-like receptor signaling in mice lacking IRAK-4**. *Nature* 2002, **416**:750-756.
65. Hoebe K, Du X, Georgel P, Janssen E, Tabet K, Kim SO, Goode J, Lin P, Mann N, Mudd S et al.: **Identification of Lps2 as a key transducer of MyD88-independent TIR signaling**. *Nature* 2003, **424**:743-748.
66. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K et al.: **Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway**. *Science* 2003, **301**:640-643.
67. Casanova JL, Abel L: **Genetic dissection of immunity to mycobacteria: the human model**. *Annu Rev Immunol* 2002, **20**:581-620.
68. Casanova JL, Abel L: **The human model: a forward genetic dissection of immunity to infection in natural conditions**. *Nat Rev Immunol* 2003, in press.