

Immune defects caused by mutations in the ubiquitin system



Amos Etzioni, MD,^{a*} Aaron Ciechanover, MD, DSc,^{b*} and Eli Pikarsky, MD, PhD^{c*}

Haifa and Jerusalem, Israel

The importance of the ubiquitin system in health and disease has been widely recognized in recent decades, with better understanding of the various components of the system and their function. Ubiquitination, which is essential to almost all biological processes in eukaryotes, was also found to play an important role in innate and adaptive immune responses. Thus it is not surprising that mutations in genes coding for components of the ubiquitin system cause immune dysregulation. The first defect in the system was described 30 years ago and is due to mutations in the nuclear factor κ B (NF- κ B) essential modulator, a key regulator of the NF- κ B pathway. With use of novel sequencing techniques, many additional mutations in different genes involved in ubiquitination and related to immune system function were identified. This can be clearly illustrated in mutations in the different activation pathways of NF- κ B, which result in aberrations in production of various proinflammatory cytokines. The inherited diseases typically manifest with immunodeficiency, autoimmunity, or autoinflammation. In this perspective we provide a short description of the ubiquitin system, with specific emphasis given to its role in the immune system. The various immunodeficiency conditions identified thus far in association with defective ubiquitination are discussed in more detail. (*J Allergy Clin Immunol* 2017;139:743-53.)

Key words: Ubiquitin, nuclear factor κ B, immunodeficiency

Degradation of a protein through the ubiquitin proteasome system (UPS) proceeds through 2 major steps: conjugation of

Abbreviations used

CVID: Common variable immunodeficiency
 DUB: Deubiquitinating enzyme
 EDA-ID: Ectodermal dysplasia with immunodeficiency
 HECT: Homologous to EGAP C-terminus
 HOIL-1: Heme-oxidase iron responsive element binding protein 2-ubiquitin ligase 1
 HOIP: HOIL-1-interacting protein
 I κ k: I κ B kinase
 KPC: KIP1 ubiquitination promoting complex
 LUBAC: Linear ubiquitin chain assembly complex
 NEMO: NF- κ B essential modulator
 NF- κ B: Nuclear factor κ B
 NIK: NF- κ B-inducing kinase
 NK: Natural killer
 PTS: Pseudo-TORCH syndrome
 RING: Really interesting new gene
 Sharpin: SH3 and multiple ankyrin repeat domains protein-associated RBK1 homology domain interacting protein
 TORCH: Toxoplasmosis, others, rubella, cytomegalovirus, herpes
 UPS: Ubiquitin proteasome system
 USP18: Ubiquitin-specific peptidase 18
 WES: Whole-exome sequencing

ubiquitin to the target substrate and degradation of the tagged protein by the 26S proteasome (Fig 1).¹ Conjugation is mediated by a cascade of 3 enzymes that act in concert. Ubiquitin is first activated by the ubiquitin-activating enzyme E1 in a reaction that requires metabolic energy. The high-energy charged ubiquitin moiety is then transferred to the ubiquitin carrier protein E2 (known also as ubiquitin-conjugating enzyme). From there, it is transferred to the target substrate that is bound to one of many specific ubiquitin ligases, E3s. It can be transferred to the substrate directly in case E3 is a member of the really interesting new gene (RING) finger domain family or through another high-energy, labile intermediate on the ligase in case the enzyme is of the homologous to EGAP C-terminus (HECT [homologous to the C-terminus of E6-AP]) domain-containing family.²

There are approximately 800 ligases in the human genome, most of them belonging to the RING finger-containing domain family, fewer to the HECT domain family, and even fewer to other smaller families. The necessity for such a large number of ligases is due to the high specificity and selectivity of the system, in which only proteins that have to be degraded will be ubiquitinated, sparing the remaining cellular proteome.

For recognition by the 26S proteasome, proteins typically have to be polyubiquitinated, during which a ubiquitin chain is typically synthesized on the ϵ -NH₂ group of an internal lysine

From ^aRuth Children Hospital, Rambam Health Campus, and ^bthe Technion Integrated Cancer Center (TICC), Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, and ^cthe Lautenberg Center for Immunology and Cancer Research, Institute for Medical Research Israel Canada (IMRIC), Hebrew University-Hadassah Medical School, Jerusalem.

*The authors contributed equally to this work.

Research in A.C.'s laboratory is supported by grants from the Israel Science Foundation (ISF), the I-CORE Program of the Planning and Budgeting Committee and the ISF (Grant1775/12), and the Deutsch-Israelische Projektkooperation (DIP). A.C. is an Israel Cancer Research Fund (ICRF) USA Professor. Research in E.P.'s laboratory is supported by grants from the European Research Council (ERC-StG livermicroenv), the Israel Science Foundation, and the DKFZ-MOST collaboration in cancer research. Both A.C. and E.P. are supported by grants from the Dr Miriam and Sheldon G. Adelson Medical Research Foundation (AMRF).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication August 23, 2016; revised October 19, 2016; accepted for publication November 29, 2016.

Corresponding author: Amos Etzioni, MD, Ruth Children Hospital, Rambam Medical Campus Bat-Galim, Haifa 31096, Israel. E-mail: etzioni@rambam.health.gov.il.

Ⓜ The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/\$36.00

© 2017 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2016.11.031>

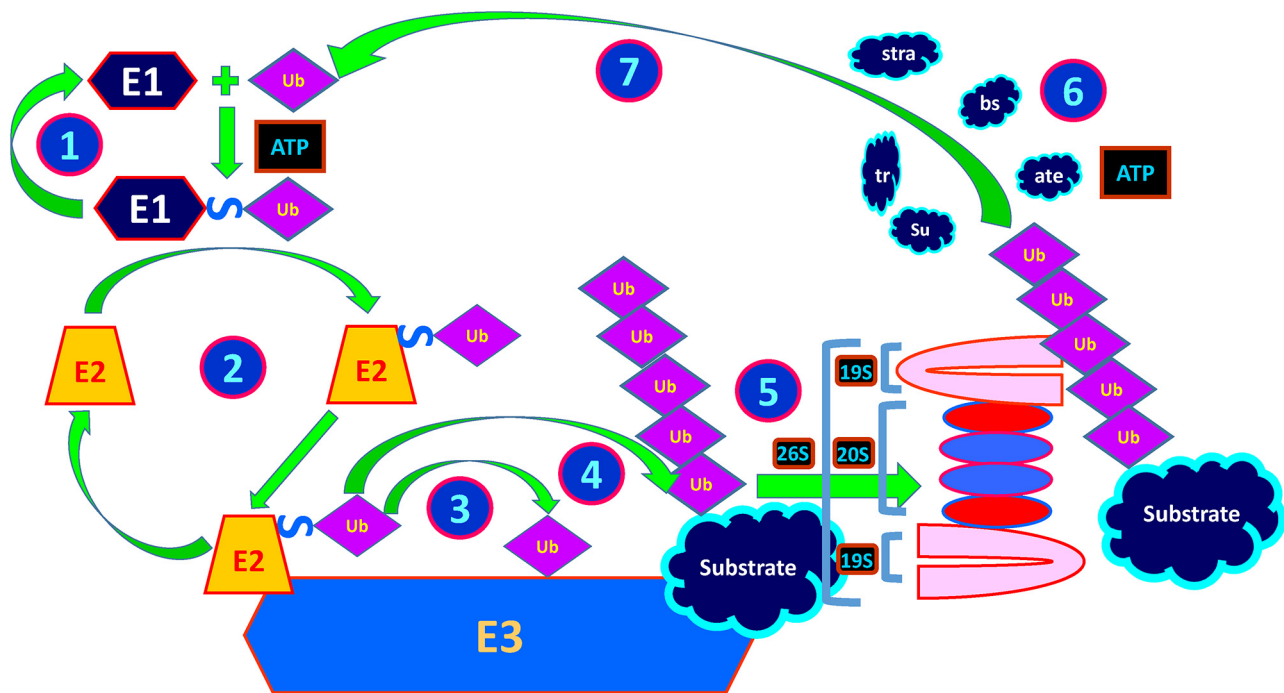


FIG 1. UPS. 1, Energy-dependent activation of ubiquitin to a high-energy intermediate on the ubiquitin-activating enzyme E1. 2, Transfer of the high-energy ubiquitin intermediate to the ubiquitin carrier protein E2 (termed also ubiquitin-conjugating enzyme [UBC]). 3 and 4, Covalent conjugation of the activated ubiquitin to the ubiquitin ligase (E3)-bound substrate either through an additional high-energy intermediate on the E3 (3; in case the E3 is of the HECT-domain group) or directly to the substrate (4; in case the E3 is of the RING finger domain group). The first ubiquitin moiety is conjugated to the substrate, and the following ubiquitins are conjugated to one another to generate a polyubiquitin chain that is the 26S proteasome-recognizing proteolytic signal. 5, Binding of the polyubiquitinated substrate to the 26S proteasome. 6, Degradation of the substrate by the 26S proteasome to short peptides, with release of intact and reusable ubiquitin (7), a reaction catalyzed by DUBs (known also as ubiquitin-specific proteases [USPs]).

residue of the substrate, and the moieties are linked to one another through their C-terminal glycine bound to an internal lysine 48 of the previous ubiquitin moiety (Fig 1).³ The polyubiquitinated protein is recognized by ubiquitin-binding subunits of the 19S regulatory complex of the 26S proteasome, and the substrate is then translocated to the 20S catalytic particle and degraded to short peptides with the release of reusable ubiquitin (Fig 1).^{3,4} These short peptides are presented on the cell surface by MHC class I molecules. Cells presenting peptides derived from foreign proteins, such as viral proteins, are recognized by cytotoxic T lymphocytes and lysed.

Similar to phosphorylation, which serves as a binding element for downstream effectors that change the fate of the modified proteins, covalent conjugation of ubiquitin or ubiquitin-like proteins to target substrates results in subsequent binding in trans of proteins with ubiquitin-binding characteristics that also affect the fate of the conjugated protein. These effectors can alter the stability, localization, activity, or function of the conjugated protein. The best studied binder is the 26S proteasome that degrades ubiquitinated proteins (Fig 1).⁴ However, in contrast to phosphorylation, which is a simple modification by a small moiety, the ubiquitin code is far more complicated. Ubiquitin itself can modify the target substrate once (monoubiquitination) or several times (multiple monoubiquitinations) or generate chains of different lengths in which one ubiquitin moiety is conjugated to the other (oligoubiquitination and polyubiquitination). Also, the chain links can be bound to one another through one of 7

internal lysine residues in the ubiquitin molecule. The ubiquitin code evolved as the different modes of modification serve different functions.⁵

The UPS contains close to 1500 components, most of them involved in specific substrate recognition. Therefore it has not been surprising to find that modification of proteins by ubiquitin and ubiquitin-like proteins is involved in basically all cellular processes. Among them are cell growth and differentiation, cell cycle and division, maintenance of the integrity of the proteome and genome, autophagy, apoptosis, regulation of transcription, signal transduction, receptor-mediated endocytosis, and protein secretion. Consequently, it has been shown that aberrations in the ubiquitin system underlie the pathogenesis of numerous diseases, with inflammatory disorders, neurodegeneration, and malignant transformation among them (Fig 2). Involvement of the ubiquitin system in disease pathogenesis is due to 2 different processes: increased production of disease enhancers, such as NF- κ B, or decreased production/presentation of disease inhibitors, such as viral antigens (Fig 2).^{6,7}

Importantly, these mechanisms have resulted in the development of several efficient drugs that target the UPS, mostly to combat malignancies. Many more are already in the pipeline.^{8,9} One group of such drugs include the proteasome inhibitors carfilzomib and ixazomib. These drugs lead to accumulation of abnormal/misfolded secretory immunoglobulins in the endoplasmic reticulum that would have otherwise been degraded through the endoplasmic reticulum-associated degradation

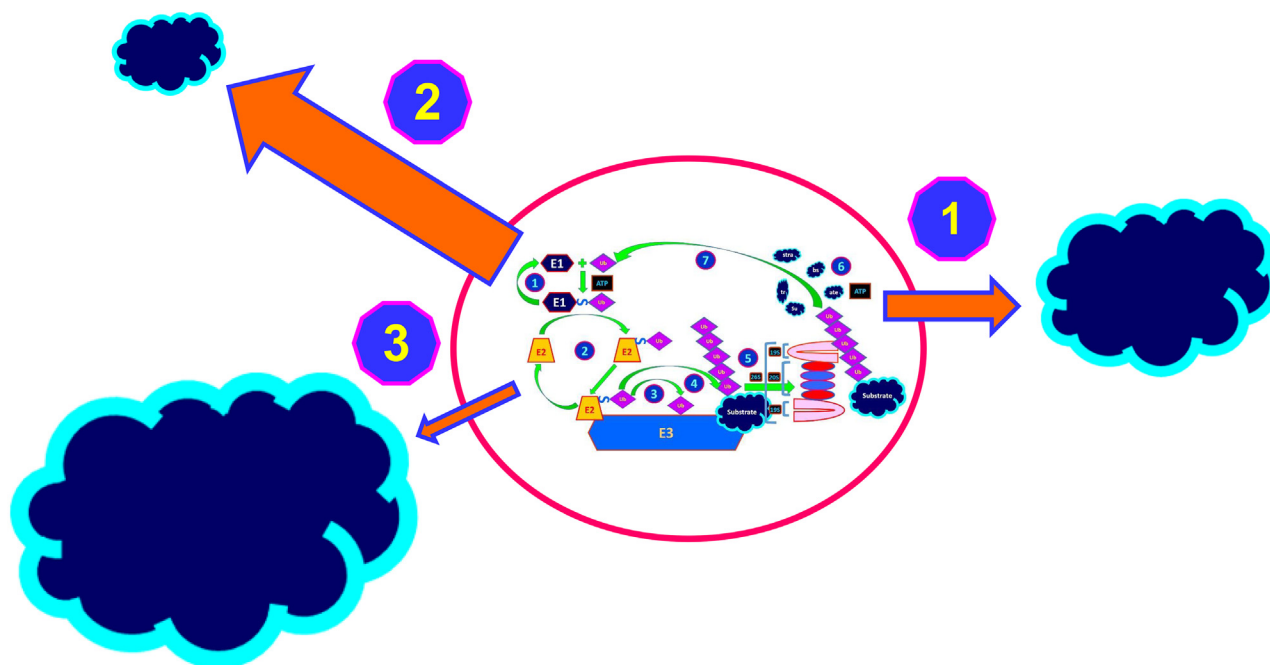


FIG 2. UPS and pathogenesis of human diseases. In case ubiquitination targets a protein for degradation, pathology can arise in one of 2 cases: excessive degradation or inhibited degradation. 1, Degradation of a substrate by a normally functioning UPS. 2, Excessive degradation of a protein decreases its level to less than its normal steady state. For example, 2 of the coded proteins of the cytomegalovirus coded proteins US2 and US11 target MHC class I molecules for ubiquitin-mediated degradation, thus inhibiting the cell's ability to present viral antigens. 3, Decreased degradation of a protein by the UPS increases its level to greater than its normal steady state. For example, the Epstein-Barr protein EBNA-1, a master transcription factor of the viral genome, contains a long Gly-Ala repeat that prevents its degradation by the ubiquitin system. Thus the stable protein, which escapes degradation, plays a key role in the long-term dormancy of the virus, securing chronic infection that might flare up in response to different stimuli.

machinery. Their accumulation results in eliciting the unfolded protein response (UPR), with subsequent induction of apoptosis.¹⁰ Another family of drugs is derived from thalidomide. These drugs serve as molecular glues, bringing together a ubiquitin ligase, in this case cereblon and a target substrate (eg, a tumor promoter, such as c-Myc or cyclin D), resulting in ubiquitination and degradation of the substrate that otherwise would not have been recognized by the UPS.¹¹

As noted, the best studied process involving the ubiquitin system is targeting substrates for degradation by the 26S proteasome. There are 3 main reasons why proteins should be degraded. First is maintenance of quality control of the proteome. Proteins are sensitive to misfolding and inactivation as a result of the effects of temperature, oxygen, mutations, and posttranslational modifications (eg, nitrosylation). Second is removal of proteins, such as cell-cycle regulators or transcription factors, which completed their function and are not necessary anymore. Third is removal of proteins during differentiation and speciation of cells and tissues.

In this review we summarize what is currently known on the involvement of aberrations of the ubiquitin system and its proteolytic arm in the pathogenesis of an important group of disorders: those of the immune and inflammatory systems.

ROLE OF UBIQUITIN IN THE IMMUNE RESPONSE

The immune system is an intricately orchestrated assembly of immune cells and their effector molecules that is poised to protect

the host against invading pathogens. In addition to its classical roles, it also participates in other homeostatic processes, including development, maturation, and aging, and plays both protective and pathologic roles in multiple disease processes, including autoimmune and autoinflammatory disorders; metabolic, endocrine, and neurodegenerative diseases; and malignancies.

The UPS is intimately associated with both innate and adaptive immune responses through its pivotal roles in the regulation of immune tolerance, immune cell development, B- and T-cell differentiation, antigen- and/or cytokine-induced intracellular signaling pathways, and hematopoiesis. The UPS is a major regulator of immune cell function, participating in the regulation and execution of essentially all key immune functions. Among these are the following (1) major signaling pathways that activate innate and adaptive immune cells; (2) pathogen recognition pathways; (3) microbial autophagy; (4) Antigen presentation; and (5) regulation of B- and T-cell receptor responses.¹²

Clear-cut evidence for the critical importance of the UPS in immune regulation are the multiple examples of pathogens that have evolved mechanisms to interfere with the UPS or else harness it for degradation of antipathogen proteins.¹³ This notion is further highlighted by the fact that bacteria are devoid of an endogenous UPS.¹⁴

One example of a major immune signaling hub is the NF- κ B signaling pathway (Fig 3). Although transcriptional programs regulated by NF- κ B are essential for the development and maintenance of multiple systems, it is perhaps better known as a

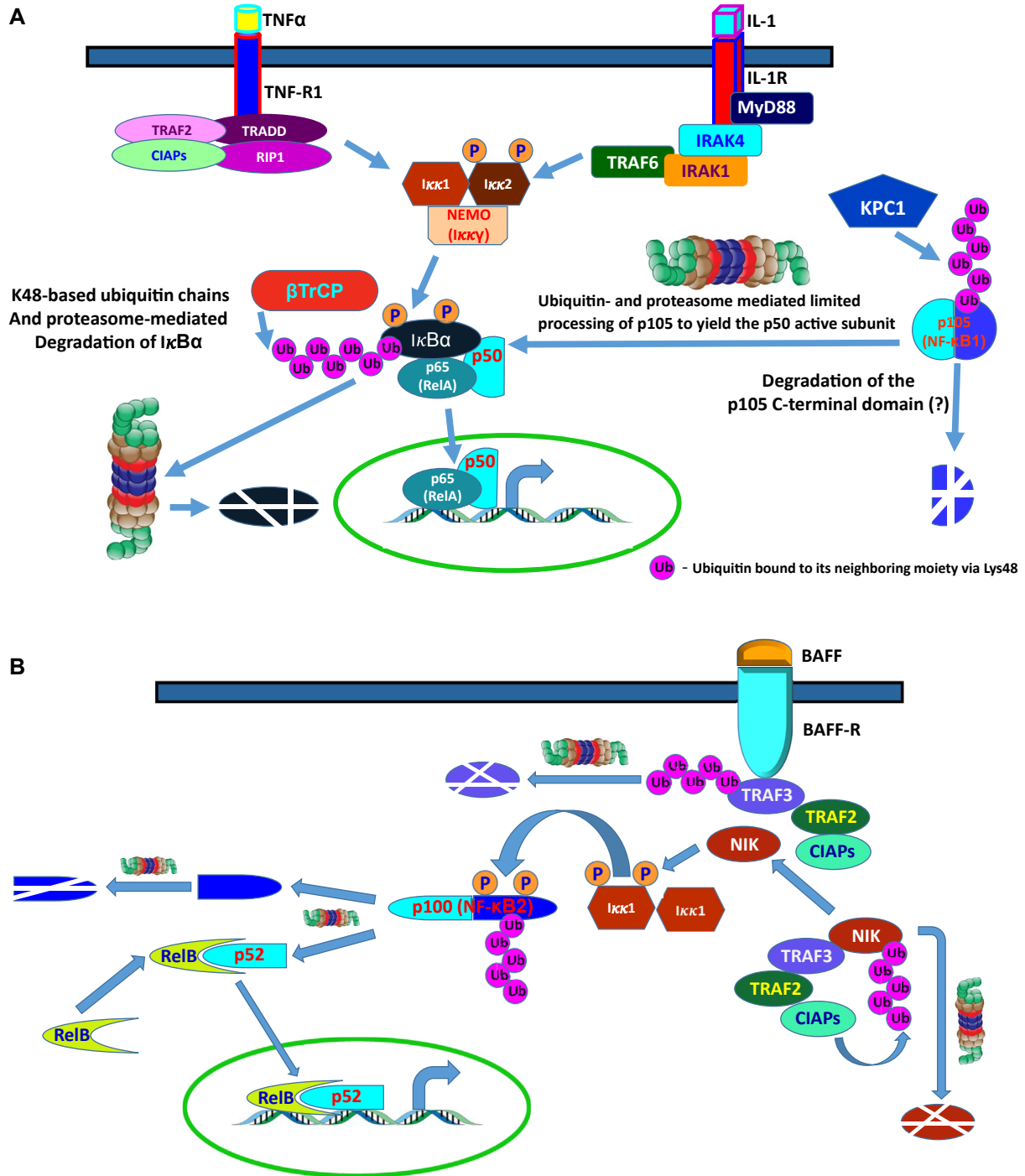
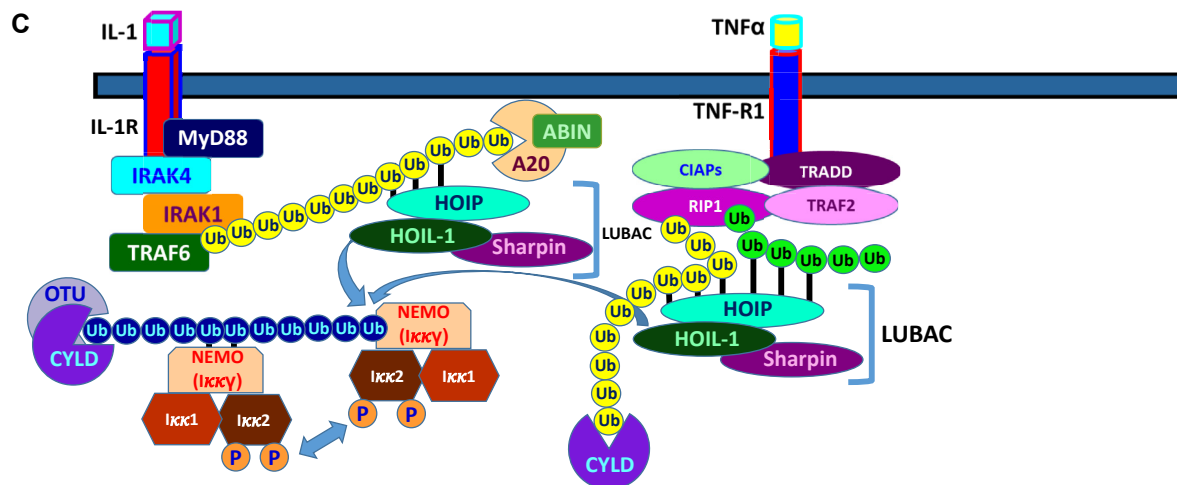
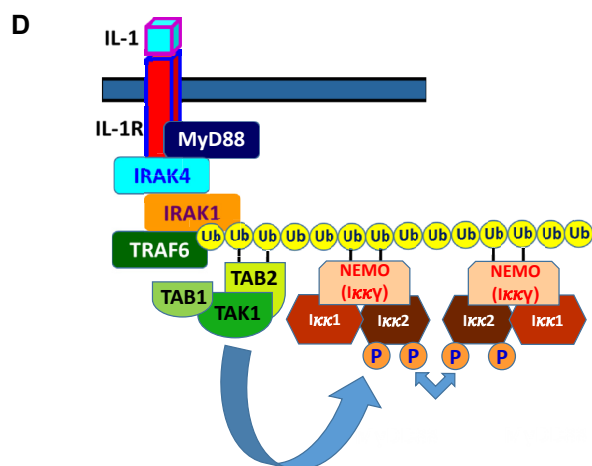


FIG 3. Ubiquitin system-mediated activation pathways of NF- κ B. **A**, The canonical pathway. In response to engagement of TNF- α or IL-1, their receptors recruit TNFR1-associated death domain (*TRADD*), receptor interacting protein 1 (*RIP1*), TNF receptor-associated factor (*TRAF*), and cellular inhibitors of apoptosis (*CIAPs*) or myeloid differentiation response gene-88 (*MyD88*), IL-1 receptor-associated kinase (*IRAK*) 4, *IRAK1*, and *TRAF6*, respectively. Either of those newly generated complexes activate the inhibitor of κ B kinase (*Iκκ*) complex, where *Iκκ2* becomes phosphorylated (in addition to *Iκκ1* and *NEMO*). The activated *Iκκ* complex phosphorylates the inhibitor *IκBα*. The inhibitor is a part of a ternary complex containing also *p50* and *p65* and its role is to retain the *p50*-*p65* dimer sequestered and inactive in the cytosol. *p50* itself is generated by means of ubiquitination (mediated by the *KPC1* ligase)– and proteasome-mediated limited processing of the longer inactive precursor, *p105*. The phosphorylated inhibitor recruits the ubiquitin ligase β TrCP, which generates K48-based chains on *IκBα*, resulting in its degradation by the proteasome. *p50*-*p65* is released and translocated to the nucleus to initiate a transcriptional program. **B**, The noncanonical pathway. *NIK* is degraded constitutively by the proteasome after ubiquitination by *CIAPs* (lower right corner). In response to engagement of B cell-activating factor (*BAFF*) with its receptor, *TRAF3* is ubiquitinated and degraded, thus removing the *CIAPs* away from *NIK*, leading to its stabilization. *NIK* phosphorylates and activates the *Iκκ1* complex, which in turn phosphorylates *p100*. This leads to ubiquitination of *p100*, followed by its proteasomal cleavage to yield



- Ubiquitin bound to its neighboring moiety via Lys63
- Linear ubiquitin chain where the moieties are bound 'head-to-tail'
- Ubiquitin bound to its neighboring moiety via Lys11



p52. p52 is then joined by RelB, and the dimer p52.RelB is translocated to the nucleus to initiate a transcriptional program. **C**, Role of M1 (linear)-, K63-, and K11-based ubiquitin chains in I κ B activation in the canonical pathway of NF- κ B. Engagement of IL-1 with its receptor (see also Fig 3, A) results in TRAF6-mediated generation of K63-based ubiquitin chains anchored both to itself (self-ubiquitination) and IRAK1. Similarly, engagement of TNF with its receptor results in generation of K63- and K11-based polyubiquitin chains anchored to RIP1. Both the K63 and K11 chains recruit the linear ubiquitin chain assembly complex made of HOIL-1, HOIP, and Sharpin, which synthesizes linear head-to-tail ubiquitin chain(s) anchored to NEMO. Binding of the I κ B complex to the linear chain either activates it directly through an allosteric effect, for example, or leads to recruitment of another I κ B complex, and the 2 transphosphorylate one another. Cyldromatosis (CYLD) and otulin (OTU) are 2 specific DUBs that cleave K63-based and linear chains. CYLD can catalyze both activities by using different substrates, such as NEMO, Bcl-3, TGF beta activated kinase (TAK) 1, and RIP1, whereas otulin can cleave only linear chains. Another player is A20, which is a K63-specific cleaving DUB that seems to be specific for TRAF6. A20 has also E3 ligase activity: after removal of the K63 chains from TRAF6, it generates K48-based chains, thus targeting it for proteasomal degradation. Together, these 3 proteins, through their DUB and ligase activities, attenuate/fine tune the cytokine-induced activation of NF- κ B. Note that A20 can bind A20-binding inhibitor (ABIN), which inhibits its activity. Thus both positive and negative effects regulate the response along the canonical pathway of NF- κ B activation. **D**, Role of K63-based ubiquitin chains in I κ B and NF- κ B canonical activation pathway through the IL-1 receptor. On engagement of IL-1 (but not TNF) with its receptor and formation of MyD88, TRAF6, and the IRAK1 and IRAK4 complex, TRAF6 synthesizes K63-based chains both on itself and on IRAK1. The chain recruits the TAK-binding protein (TAB) 1, TAB2, and TAK1 to generate a kinase complex and the NEMO-based I κ B complex. The I κ B complex is now activated by the TAK-TAB complex and/or by *trans* cross-phosphorylation. Ub, Ubiquitin.

major immune regulator. Indeed, several inherited diseases that involve different components of this pathway are manifested by immune dysfunction. NF- κ B activation is governed by a number of positive and negative regulatory elements (Fig 3). In the resting state NF- κ B dimers are held inactive in the cytoplasm through association with I κ B proteins. Inducing stimuli trigger activation of the I κ B kinase complex, leading to phosphorylation, ubiquitination, and degradation of I κ B proteins.

Released NF- κ B dimers translocate to the nucleus, bind specific DNA sequences, and promote transcription of target genes.¹⁵ The 2 main regulators of this classic NF- κ B signaling pathway are TNF and IL-1. This stepwise process is considered a classic example of phosphorylation-dependent degradation by the UPS.¹⁶

Signal-induced degradation of I κ B is not the only step in which ubiquitination and degradation are involved in the NF- κ B signaling pathway. An important step is stabilization of the TNF receptor 1 signaling complex mediated by linear ubiquitination. This mode of modification is crucial for innate and adaptive immune signaling.¹⁷ The linear ubiquitin chain assembly complex (LUBAC) catalyzes head-to-tail assembly of ubiquitin moieties. Recruitment of LUBAC and the linear ubiquitination of NF- κ B essential modulator (NEMO) stabilizes the TNF receptor 1 signaling complex and is required for TNF-mediated NF- κ B activation. Recruitment of LUBAC occurs after ligand binding to the receptor and is important for facilitating the downstream classical ubiquitination. In the absence of LUBAC components, cells display reduced TNF-induced NF- κ B activation.¹⁸

The termination of an immune response is as important as its initiation. This process is mediated by deubiquitination, in which ubiquitin chains are deconjugated by a family of deubiquitinating enzymes (DUBs). The human genome encodes approximately 100 different DUBs having thus substrate selectivity and functional specificity. For example, mutations in the tumor suppressor DUB cylindromatosis were found to cause benign tumors (cylindromas) of skin appendages. The enzyme was found to have an important role in regulation of NF- κ B by removing K63-linked ubiquitin chains from I κ B kinase signaling components, such as TNF receptor-associated factor 2 and NEMO (NF- κ B essential modifier, which is known also as I κ B kinase [I κ K] γ), thus inhibiting NF- κ B pathway activation.¹⁹

As noted above, I κ B α and other inhibitory proteins in the pathway are degraded after phosphorylation and ubiquitination. Importantly, the inactive precursor proteins NF- κ B1 (p105) and NF- κ B2 (p100) are activated through UPS-mediated limited processing to the respective NF- κ B active subunits p50 and p52. In the case of p105, after ubiquitination, the C-terminal domain is degraded by the proteasome, yielding p50.^{20,21} This is a rather unique example in which a protein enters the proteasome yet is not fully degraded. The resulting p50 subunit can further heterodimerize with members of the REL family of proteins, generating the active NF- κ B transcription factor itself. Proteasomal processing of p105 occurs primarily under basal conditions and requires prior ubiquitination by the KIP1 ubiquitination promoting complex (KPC), a heterodimer made of KPC1 (RNF123) and KPC2 (UBAC1).²²

With the advance in understanding of the crucial role of the ubiquitin system in the immune response and the use of new genetic tools, such as whole-exome sequencing (WES), defects in

genes that are part of the UPS were found to result in immune deficiency conditions, many of them involving the NF- κ B signaling pathway (Fig 3 and Table I).

IMMUNODEFICIENCY CAUSED BY INHERITED IMPAIRMENT IN THE UPS

What follows is a list of inherited diseases that involve mutations in the UPS and result in immunodeficiency. Remarkably, the vast majority of these mutations affect the NF- κ B pathway, attesting to the crucial role of the UPS in regulation of this key immune hub and the pivotal role of NF- κ B in immune regulation.

X-linked ectodermal dysplasia with immunodeficiency

Perhaps the first immune defect directly linked to an aberration in the UPS (and also the NF- κ B activation pathway) was reported in 1986 in a young boy with anhidrotic ectodermal dysplasia who eventually died from miliary tuberculosis.²³ It took an additional 10 years to describe the immune abnormalities associated with this condition, which is known today as ectodermal dysplasia with immunodeficiency (EDA-ID).²⁴

In 2000, it was found that in most cases the disease is caused by a hypomorphic mutation in NEMO located on the X-chromosome, which is a major constituent of the inhibitor of κ B kinase complex (Fig 1).²⁵ The defect occurs in 1:250,000 live male births. These children experience life-threatening infections combined with abnormal development of tissues originating from the ectoderm.²⁶ They have conic teeth, frontal bossing, and sparse scalp hair with dry skin caused by the absence of sweat glands. From early life, they experience severe bacterial infections and rarely fungal or mycobacterial infections.²⁶ About 25% of the patients have inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel disease. More than half of the patients will die during childhood, with a mean age of death of 6 years.²⁷ Their immune deficiency is mainly caused by T- and B-cell dysfunction. Hypogammaglobulinemia with lack of polysaccharide-specific antibodies is typically noted. In some cases a hyper-IgM-like phenotype was observed.²⁸ Impaired natural killer (NK) cell cytotoxicity with normal NK cell numbers is common, and reduced LPS and IL-1 family protein responses were also found. In about 10% of the cases, a reduced mitogen-induced proliferation of T lymphocytes was described.²⁹

Almost 50 different mutations were described that are scattered throughout the NEMO gene, and these account for the varying clinical phenotype. Indeed, genotype/phenotype correlation does exist to some extent. These mutations are hypomorphic because they lead to an impairment of NF- κ B signaling but not to its abolition. Most mutations cause impaired protein expression, defective folding, or both.³⁰ In some cases a missense mutation does not impair NEMO synthesis or folding but manifests a defect in the polyubiquitin chain synthesis on NEMO, resulting in a similar phenotype seen in a dysfunctional protein.³¹ Interestingly, in many cases spontaneous reversion mosaicism of the NEMO gene was found. In one study 9 of 10 patients had reversal mutations.³² This phenomenon was observed mainly in the T-cell lineage. This might point to the fact that NEMO expression is critical for T-cell survival but less crucial for B-cell and

TABLE I. Immunodeficiency caused by defects in the ubiquitin system

Name	Gene defect	Inheritance	Functional defect	Immunologic findings	Nonimmunologic features	OMIM ID
XL-EDA-ID	NEMO (LOF)	XL	Impaired NF- κ B signaling	Hypogammaglobulinemia with defective specific antibody response with hyper-IgM; impaired cytotoxicity, mild T-cell defect	Abnormal development of tissues originating from the ectoderm	300291
AD-EDA-ID	NF- κ B1a (GOF)	AD	Impaired phosphorylation of I κ B α ; denying entrance to the nucleus	Profound combined immunodeficiency + autoimmunity	Like XL-EDA	612132
IKK2 deficiency	I κ B κ B (LOF)	AR	Defective canonical NF- κ B signaling pathway	Severe combined immunodeficiency		615592
NF- κ B-CVID	NF- κ B1 and NF- κ B2 (LOF)	AD or haploinsufficiency	Defective processing of p100 to p52 impaired ubiquitination	CVID	Adrenal insufficiency and alopecia areata	615577 616576
LUBAC deficiency	HOIL-1 or HOIP (LOF + GOF)	AR	Defective linear ubiquitination caused by impaired LUBAC formation	Defective response to TNF and IL-1 β ; impaired B- and T-cell function	Autoinflammation, amylopectinosis	610924
USP18 deficiency	USP18 (GOF)	AR	Defective negative regulation of type 1 interferon signaling	Increase accumulation of type I interferon	TORCH-like syndrome	607057
A20 haploinsufficiency	TNFAIP3 (GOF)	Haploinsufficiency	Defective inhibition of NF- κ B signaling pathway	Autoantibodies	Arthralgia, mucosal ulcers, ocular inflammation	616744
NIK deficiency	MAP3K14 (LOF)	AR	Defective noncanonical NF- κ B signaling pathway	Profound B-cell defect with T-cell and NK cell abnormalities		604655
Otulipenia/ORAS	Otulin (LOF)	AR	Increase LUBAC induction of NK- κ B activation	High proinflammatory cytokines levels	Fever, diarrhea, dermatitis	615712

GOF, Gain of function; LOF, loss of function; OMIM, Online Mendelian Inheritance in Man; ORAS, otulin-related autoinflammatory syndrome; XL, X-linked.

monocyte survival. The somatic mosaicism in T cells might thus be an important factor leading to the various inflammatory disorders seen in patients with X-linked EDA-ID. Complete loss-of-function mutations in NEMO caused by a stop codon, for example, are lethal *in utero* in male subjects and lead to X-linked dominant incontinentia pigmenti in female subjects. Although immunoglobulin substitution and antibiotic treatment are of help, the only way to correct the defect is stem cell transplantation.³³

Autosomal dominant EDA-ID

Rarely, EDA-ID is inherited in an autosomal dominant form. This was first described in 2003 and is caused by a hypermorphic mutation of the *NFKB1A* gene located on chromosome 14, which encodes the major inhibitory protein of the NF- κ B pathway, I κ B α .³⁴ All the mutations had a gain-of-function effect. Seven different mutations have been identified thus far. The mutations enhance the inhibitory capacity of I κ B α , thus interfering with its phosphorylation and subsequent degradation, resulting in severely impaired NF- κ B activation.³⁵ Accordingly, reduced IL-6 and IL-10 production by leukocytes in response to NF- κ B-dependent stimulation was observed.³⁶

A profound combined immunodeficiency can be observed from early in life. Patients have decreased T-cell numbers and function, as well as hypogammaglobulinemia with no production of specific antibodies. Early on, they have severe and invasive

pyogenic bacterial infections but can also experience fungal or mycobacterial infections. Furthermore, they can have several autoimmune disorders and in one case had polyendocrinopathy. Although the clinical symptoms in patients with autosomal dominant EDA-ID are similar (or a bit more severe) to those observed in patients with X-linked inheritance, patients with autosomal dominant EDA-ID do not have any cytotoxic defect, as observed in the X-linked form, but lack memory T cells that do exist in patients with X-linked EDA-ID.²⁶

With regard to therapy, patients with autosomal dominant EDA-ID should start immunoglobulin supplementation early in life combined with antibiotic and antifungal prophylaxis. In several cases of both forms of EDA-ID, stem cell transplantation was attempted with mixed results, implying that these patients might have some intrinsic difficulties with successful engraftment.³⁷

Autosomal recessive I κ k2 deficiency

This is a rare immunodeficiency syndrome that has been described only in 10 patients thus far. It is caused by mutations in the *IKBKB* gene located on chromosome 8, which encodes for inhibitor of κ B kinase 2, the central catalytic component of the I κ k complex in the canonical NF- κ B signaling pathway (Fig 3, A, C, and D).³⁸ In all patients homozygous nonsense mutations were identified, leading to lack of expression of the I κ k2 protein.³⁹ Although in some patients the NEMO level was normal, in others

it was decreased. All the patients had hypogammaglobulinemia with clinical characteristics of severe combined immunodeficiency, including early onset of severe viral, bacterial, and fungal infections despite normal numbers of T and B lymphocytes. However, they have low numbers of regulatory T and NK cells.^{40,41} All T and B cells were of the naive phenotype. Immune cells showed impaired response to stimulation through their different receptors. Thus both adaptive and innate immune responses were defective, leading to a severe clinical presentation.⁴⁰ Thus, although I κ B2 seems dispensable for the development of T and B lymphocytes and NK cells in human subjects, it is indispensable for their activation.⁴⁰

Of note, the nonimmunologic features observed in patients with other defects in the NF- κ B pathway were absent in patients with I κ B2 deficiency.³⁸ This suggests that I κ B2 is dispensable for skin adnexal development. Most likely, the catalytic activity of I κ B1 is sufficient to compensate for the absence of I κ B2 in this setting. All patients were treated with antibiotics and gamma globulin supplementation. Seven of the 10 patients underwent stem cell transplantation, but only 2 survived.

NF- κ B1 and NF- κ B2: Common variable immunodeficiency

Common variable immunodeficiency (CVID) is a heterogeneous group of conditions leading to hypogammaglobulinemia and recurrent infections. Although the pathogenetic mechanisms in the majority of patients are still obscure, an impaired canonical NF- κ B signaling in B cells of patients with CVID is quite common.⁴² Because in most cases the genetic basis is unknown, WES is required to reveal the genetic defects in patients with this condition. By using WES, several cases of CVID were found to be caused by mutations in NF- κ B2 and NF- κ B1.³⁸ Recently, it was found that of 320 patients with CVID, 33 mutations in NF- κ B1 or NF- κ B2 were identified, accounting for approximately 10% of monogenetic defects in patients with CVID (B. Grimbacher, personal communication). Germline mutations in NF- κ B2 as a cause for CVID were first described in 2013 in 2 families with early-onset hypogammaglobulinemia with variable autoimmune features, as well as adrenal insufficiency.⁴³ Two different autosomal dominant mutations that affect the C-terminus of the protein were found. These heterogeneous mutations introduce a premature stop codon in a region required for phosphorylation and ubiquitination. Accordingly, reduced activation and nuclear translocation were found.⁴³

Because some p52 (see above) still exists in these patients, implying the presence of a low amount of wild-type p100, it was suggested that CVID pathogenesis in patients with heterogeneous NF- κ B2 mutations results from haploinsufficiency and is not due to a dominant negative effect.⁴³ Still, in a more recent study another mutation in the gene (D865G) was found to have an autosomal dominant effect, leading to absence of circulating B cells.⁴⁴ This mutation resulted in failure of p100 phosphorylation, which blocks processing to p52. The severe B-cell deficiency appears to be due to disruption of both the canonical (NF- κ B1; Fig 3, A) and noncanonical (NF- κ B2; Fig 3, B) pathways by the mutant p100. Also, in this study the patients experienced alopecia totalis and central adrenal insufficiency, the cause of which is still unknown.⁴⁴

Looking further in families with CVID, mutations in the NF- κ B1 gene were also found in 3 families from different genetic backgrounds.⁴⁵ The mutations caused in-frame deletion of exons 8 or 9 or premature termination of translation. This form of CVID is due to NF- κ B1 p50 haploinsufficiency. Another patient with NF- κ B1 haploinsufficiency was described in whom, aside from the immunodeficiency, severe EBV-associated lymphoproliferative disease was observed that responded to rituximab.⁴⁶

As opposed to the broad functions and numerous ligands of the canonical (NF- κ B1) pathway that led to the production of numerous inflammatory cytokines, the noncanonical pathway mostly involves the B-cell lineage.⁴⁷ Therefore it is not surprising that in all of the first 4 described cases of mutations in NF- κ B2, immunoglobulin levels were reduced along with reduced memory and marginal zone B-cell counts. The patients responded poorly to immunization. Of some surprise was the finding of normal levels of circulating B cells.⁴³

In another patient it was found that although expression of the various activating and inhibitory NK receptors was normal, a defective NK cell cytotoxic activity was noted.⁴⁸ This might explain the severe viral infections seen in this group of patients, which are not common in other patients with CVID.

LUBAC deficiency

As noted above, LUBAC is an important multiprotein complex that generates linear chains of ubiquitin on protein substrates. It is composed of 3 proteins: HOIL-1-interacting protein (HOIP), the actual ligase; heme-oxidase iron responsive element binding protein 2-ubiquitin ligase 1 (HOIL-1); and SH3 and multiple ankyrin repeat domains protein-associated RBK1 homology domain interacting protein (Sharpin). In recent years, mutations in these components were linked to various clinical presentations.⁴⁹

Some patients with HOIL-1 mutations exhibit cardiomyopathy, amylopectinosis, autoinflammation, and immunodeficiency, whereas others did not have any kind of immunodeficiency.⁵⁰ This difference might be due to the nature and location of the HOIL-1 mutation or to other environmental (eg, persistent viral infection) factors. Boisson et al⁵¹ described 3 children with a fatal inherited disorder characterized by chronic autoinflammation, invasive bacterial infections, and muscular amylopectinosis. They all carried biallelic loss of HOIL-1 (encoded by the *RBCK1* gene). In this autosomal recessive condition the mutations resulted in impairment of LUBAC stability. What was puzzling in this situation was the association of systemic autoinflammation with immune deficiency. As expected, impaired NF- κ B activation resulted in impaired response to TNF and the amount of IL-1 β generated, as manifested by a defect in humoral immunity with deficiency of memory B cells and impaired antibody response to polysaccharide antigens.⁵¹

On the other hand, the autoinflammatory phenotype is consistent with the fact that leukocytes, mainly monocytes, displayed constitutive overexpression of numerous inflammatory genes and were hyperresponsive to IL-1 β . This paradoxical enhanced response to the cytokine might be due to impairment in proteasomal degradation of 1 or more proinflammatory mediators or to impairment of a negative regulatory mechanism. All in all, it seems that HOIL-1 deficiency has different effects on different types of leukocytes.⁵⁰

Recently, a homozygous mutation in HOIP, the ligase component of the LUBAC complex, was identified in one child who also had both autoinflammation and immune deficiency.⁵² The missense allele was severely hypomorphic because it impaired HOIP expression and destabilized the entire LUBAC complex. The patient showed a broad impairment of antibody production and severe T-cell lymphopenia, which was not observed in patients with HOIL-1 deficiency. The patient also had systemic lymphangiectasia not seen in patients with HOIL-1 deficiency.⁵²

Ubiquitin-specific peptidase 18 deficiency

Toxoplasmosis, others, rubella, cytomegalovirus, herpes (TORCH) is a well-recognized syndrome caused by transplacental infection with several pathogens, including *Toxoplasma* species, rubella, and *Herpes* species. It affects various organs in the fetus, mainly the brain, leading to microcephaly, cerebral atrophy, and calcifications.⁵³ Cases with similar characteristics but in which no infectious agents are detected are referred to as pseudo-TORCH syndrome (PTS).⁵⁴ Because many of the patients came from families with consanguinity, it was reasonable to believe that the syndrome is due to an underlying genetic defect. Indeed, another similar condition, Aicardi-Goutieres syndrome, results from increased accumulation of type 1 interferon and is due to constitutive activation of the interferon signaling pathway.⁵⁵ Several patients with PTS were identified to carry mutations in ubiquitin-specific peptidase 18 (*USP18*),⁵⁶ which is a key negative regulator of type 1 interferon signaling.⁵⁷ *In vitro* experiments with fibroblasts isolated from 5 patients having this loss of function recessive mutation displayed severely enhanced interferon-induced inflammation.⁵⁶ The role of interferon in patients with PTS was also demonstrated in patients with ISG15 deficiency. ISG15 is a secreted di-ubiquitin-like protein that, in addition to being able to conjugate to proteins, plays an important role in IFN- γ -induced antimicrobial immunity. Intracellularly, ISG15 is important in USP18-mediated downregulation of interferon signaling, thus suppressing type 1 interferon inflammatory response.⁵⁸ Patients with ISG15 deficiency also display a reduced level of USP18.⁵⁹ USP18 deficiency, which causes PTS, might suggest that in patients with some congenital viral infections, the brain damage is due, at least in part, to a host response (high type 1 interferon levels) and not to the virus itself. Furthermore, USP18 can serve as a therapeutic agent in patients with type 1 interferonopathies.

A20 insufficiency

Recently, a new disorder was described in which mutations in *TNFAIP3* led to severe immune dysregulation.⁶⁰ *TNFAIP3* encodes for the NF- κ B regulatory protein A20, which regulates multiple ubiquitin-dependent innate immune signaling cascades, including those downstream of TNF receptor 1, IL-1 receptor, Toll-like receptor 4, and others. Several proposed mechanisms for the inhibitory function of A20 on the UPS were suggested.⁶¹ It is known to have 2 functions. First, it cleaves K63-linked ubiquitin chains and thus reduces the affinity of NEMO to the I κ k complex. It is also known to be a ligase, synthesizing K48-based chains, which thus supports the degradation of E2 enzymes, thereby inhibiting E3 ligase activity that is dependent on these E2s.⁶² In addition, A20 directly inhibits I κ k activity independently of its deubiquitinase activity in a manner that depends on binding to NEMO.⁶³

Indeed, in 6 unrelated families with mutations in A20, increased expression of NF- κ B-induced proinflammatory cytokines were detected.⁶⁰ The patients had early-onset systemic inflammation resembling Behçet disease. They had arthralgia, oral and genital ulcers, and ocular inflammation. Although a dominant disorder was found in all 6 families, the mutant truncated A20 proteins, which caused reduced A20 expression in patients' cells, are likely to act through haploinsufficiency because they do not exert a dominant negative effect in overexpression experiments.⁴² Although mice deficient for A20 die prematurely of severe multiorgan inflammation,⁶⁴ gene targeting studies have clearly demonstrated the importance of variants in A20 in multiple cells types in the pathogenesis of inflammation and autoimmunity.³⁹

Combined immunodeficiency associated with NF- κ B-inducing kinase defects

The NF- κ B-inducing kinase (NIK) is an integral component of the noncanonical NF- κ B pathway activated by the TNF receptor family and is essential for B-cell immunity.⁶⁵ Recently, a biallelic loss-of-function mutation in *MAP3K14*, which encodes for NIK, was found in 2 patients from a large consanguineous family.⁶⁶ The gene is located on chromosome 17q21, and by using diverse genetic analysis, a single homozygous mutation (Pro565Arg) was found in the 2 patients. This residue is located within the kinase domain of the protein and is highly conserved through evolution. Although in the NIK knockout mice a humoral defect with decreased B-cell numbers and low immunoglobulins levels was observed,⁶⁷ in patients a broader combined immunodeficiency was found.⁶⁶ Indeed, a profound survival defect of B cells was observed with hypogammaglobulinemia. Still, the clinical phenotype (increased viral infections, *Cryptosporidium* species infection, and tuberculous osteomyelitis caused by dissemination after BCG vaccination) suggested a broader immune defect. Although overall T-cell numbers were normal, both follicular helper and memory T cells were perturbed, and defective response toward tetanus toxoid and tuberculin was found. Furthermore, NK cell numbers were reduced, and they exhibited defective activation.

Therefore it is clear that the noncanonical pathway is involved not only with B-cell maturation but also in various functions of other immune cells.⁶⁸ Both patients underwent hematopoietic stem cell transplantation, but the one who did not receive any conditioning died, suggesting that at least reduced-intensity conditioning is indicated for engraftment.⁶⁶

Otulin-related autoinflammatory syndrome/otulipenia

Otulin is a deubiquitination enzyme with unique specificity that antagonizes LUBAC-mediated ubiquitination. Thus it can effectively inhibit LUBAC-induced NF- κ B activation elicited by TNF, for example (Fig 3, C).⁶⁹

Recently, 2 independent groups described loss-of-function mutations in otulin in several consanguineous families. From early after birth, patients with the mutation experienced fever, diarrhea, neutrophilic dermatitis, and failure to thrive and died if specific therapy was not instituted. No overt immunodeficiency was observed, and the function of T and B lymphocytes was unaffected. Mononuclear cells showed an increase response to

signaling in the canonical NF- κ B pathway, and high levels of proinflammatory cytokines were detected in the serum during exacerbation of the disease. Serum C-reactive protein levels, neutrophil counts, and immunoglobulins levels were markedly increased, with no evidence of infection.^{70,71}

Stimulation of cells derived from patients with LPS induced high levels of IL-1 β , IL-6, and TNF. In the 2 studies it was shown that loss-of-function mutations in otulin resulted in increased linear ubiquitination of signaling molecules, resulting in increased NF- κ B activation and severe sterile inflammation.⁷¹ Interestingly enough, a mutation in another DUB, A20, which also attenuates NF- κ B activation (see above), has a less severe inflammatory phenotype.⁶⁰ This might be due to the fact that patients with otulin mutations have a more profound protein deficiency, whereas the A20 mutation leads to a haploinsufficient condition.

Although most of the patients died before the age of 3 years, in 2 patients who were treated with anti-TNF neutralizing antibodies (infliximab), a dramatic improvement was noted, and they were able to lead a normal life on continuous infliximab treatment.^{70,71} The same phenotype and response to infliximab was observed also in mouse models with mutations in otulin.⁷⁰ It should be noted that deletion of otulin is embryonically lethal and is possibly caused by its role in angiogenesis. This new condition was named otulin-related autoinflammatory syndrome⁷⁰ by one group and otulipenia by the other group.⁷¹

Novel genetic analysis techniques will likely identify other monogenetic inflammatory diseases possibly involving genes in the NF- κ B pathway, expanding our knowledge on monogenetic immune diseases.

REFERENCES

- Ciechanover A. The unravelling of the ubiquitin system. *Nat Rev Mol Cell Biol* 2015;16:322-4.
- Dove KK, Stieglitz B, Duncan ED, Rittinger K, Kleivit RE. Molecular insights into RBR E3 ligase ubiquitin transfer mechanisms. *EMBO Rep* 2016;17:1221-35.
- Ciechanover A, Stanhill A. The complexity of recognition of ubiquitinated substrates by the 26S proteasome. *Biochim Biophys Acta* 2014;1843:86-96.
- Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 2002;82:373-428.
- Akutsu M, Dikic I, Bremm A. Ubiquitin chain diversity at a glance. *J Cell Sci* 2016;129:875-80.
- Lilley BN, Ploegh HL. Viral modulation of antigen presentation: manipulation of cellular targets in the ER and beyond. *Immunol Rev* 2005;207:126-44.
- Masucci MG. Epstein-Barr virus oncogenesis and the ubiquitin-proteasome system. *Oncogene* 2004;23:2107-15.
- Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. *Nat Med* 2014;20:1242-53.
- Huang X, Dixit VM. Drugging the undruggables: exploring the ubiquitin system for drug development. *Cell Res* 2016;26:484-98.
- Teicher BA, Tomaszewski JE. Proteasome inhibitors. *Biochem Pharmacol* 2015; 96:1-9.
- Guirguis AA, Ebert BL. Lenalidomide: deciphering mechanisms of action in myeloma, myelodysplastic syndrome and beyond. *Curr Opin Cell Biol* 2015; 37:61-7.
- Jiang X, Chen ZJ. The role of ubiquitylation in immune defence and pathogen evasion. *Nat Rev Immunol* 2011;12:35-48.
- Gustin JK, Moses AV, Fruh K, Doauglas JL. Viral takeover of the host ubiquitin system. *Front Microbiol* 2011;2:161.
- Loureiro J, Ploegh HL. Antigen presentation and the ubiquitin-proteasome system in host-pathogen interactions. *Adv Immunol* 2006;92:225-305.
- Hayden MS, Ghosh S. NF- κ B, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev* 2012;26:203-34.
- Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF- κ B activity. *Annu Rev Immunol* 2000;18:621-63.
- Shimizu Y, Taraborrelli L, Walczak H. Linear ubiquitination in immunity. *Immunol Rev* 2015;266:190-207.
- Haas TL, Emmerich CH, Gerlach B, Schmukle AC, Cordier SM, Rieser E, et al. Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. *Mol Cell* 2009;36:831-44.
- Sun SC. CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB activation and diverse biological processes. *Cell Death Differ* 2010;17:25-34.
- Fan CM, Maniatis T. Generation of p50 subunit of NF-kappa B by processing of p105 through an ATP-dependent pathway. *Nature* 1991;354:395-8.
- Orian A, Whiteside S, Israël A, Stancovski I, Schwartz AL, Ciechanover A. Ubiquitin-mediated processing of NF-kappa B transcriptional activator precursor p105. Reconstitution of a cell-free system and identification of the ubiquitin-carrier protein, E2, and a novel ubiquitin-protein ligase, E3, involved in conjugation. *J Biol Chem* 1995;270:21707-14.
- Kravtsova-Ivantsiv Y, Shomer I, Cohen-Kaplan V, Snijder B, Superti-Furga G, Gonen HS, et al. KPC1-mediated ubiquitination and proteasomal processing of NF- κ B1 p105 to p50 restricts tumor growth. *Cell* 2015;161:333-47.
- Frix CD 3rd, Bronson DM. Acute miliary tuberculosis in a child with anhidrotic ectodermal dysplasia. *Pediatr Dermatol* 1986;3:464-7.
- Abinun M. Ectodermal dysplasia and immunodeficiency. *Arch Dis Child* 1995; 73:185.
- Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, et al. Xlinked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet* 2001;27:277-85.
- Kawai T, Nishikomori R, Heike T. Diagnosis and treatment in anhidrotic ectodermal dysplasia with immunodeficiency. *Allergol Int* 2012;61:207-17.
- Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ, et al. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. *J Allergy Clin Immunol* 2008;122:1169-77.
- Paciolla M, Pescatore A, Conte MI, Esposito E, Incoronato M, Lioi MB, et al. Rare Mendelian primary immunodeficiency diseases associated to impaired NK-kB signaling. *Genes Immun* 2015;16:239-46.
- Puel A, Picard C, Ku CL, Smahi A, Casanova JL. Inherited disorders of NF-kB mediated immunity in man. *Curr Opin Immunol* 2004;16:34-41.
- Fusco F, Pescatore A, Conte MI, Mirabelli P, Paciolla M, Esposito E, et al. EDA-ID and IP, two faces of the same coin: how the same IkbkG/NEMO mutation affecting the NF-kN pathway can cause immunodeficiency and/or inflammation. *Int Rev Immunol* 2015;34:445-59.
- Hubrau M, Ngadjjeu F, Puel A, Israel L, Feinberg J, Chrabieh M, et al. New mechanism of X-linked anhidrotic ectodermal dysplasia with immunodeficiency: impairment of ubiquitin binding despite normal folding of NEMO protein. *Blood* 2011;118:926-34.
- Kawai T, Nishikomori R, Izawa K, Murata Y, Tanaka N, Sakai H, et al. Frequent somatic mosaicism of NEMO in T cells of patients with X-linked anhidrotic ectodermal dysplasia with immunodeficiency. *Blood* 2012;119: 5458-66.
- Klemann C, Pannicke U, Morris-Rosendahl DJ, Vlantis K, Rizzi M, Uhlig H. Transplantation from a symptomatic carrier sister restores host defences but does not prevent colitis in NEMO deficiency. *Clin Immunol* 2016;164:52-6.
- Courtois G, Smahi A, Reichenbach J, Doffinger R, Cancrini C, Bonnet M, et al. A hypermorphic Ikbk mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest* 2003;112: 1108-15.
- Lopez-Granados E, Keenan JE, Kinney MC, Leo H, Jain N, Ma CA, et al. A novel mutation in NFKBIA/IKBA results in a degradation-resistant N-truncated protein and is associated with ectodermal dysplasia with immunodeficiency. *Hum Mutat* 2008;29:861-8.
- Schimke LF, Rieber N, Rylaarsdam S, Carbal-Marques O, Hubbard N, Puel A, et al. A novel Gain of function IkBA mutation underlies ectodermal dysplasia with immunodeficiency and polyendocrinology. *J Clin Immunol* 2013;33: 1088-99.
- Fish JD, Duerst RE, Gelfand EW, Orange JS, Bunin N. Challenges in the use of allogeneic hematopoietic SCT for ectodermal dysplasia with immune deficiency. *Bone Marrow Transplant* 2009;43:217-21.
- Senegas A, Gautheron J, Gentil A, Maurin D, Courtois G. IKK-related genetic diseases: probing NF-kB functions in humans and other matters. *Cell Mol Life Sci* 2015;72:1275-87.
- Burns SO, Plagnol V, Gutierrez BM, Al Zahrani D, Curtis J, Hasan A, et al. Immunodeficiency and disseminated mycobacterial infection associated with homozygous nonsense mutation in Ikbk. *J Allergy Clin Immunol* 2014;134: 215-8.
- Pannicke U, Baumann B, Fuchs S, Henneke P, Rensing-Ehl A, Rizzi M, et al. Deficiency of innate and acquired immunity caused by an IKBKB mutation. *N Engl J Med* 2013;369:2504-14.

41. Mousallem T, Yang J, Urban TJ, Wang H, Adeli M, Parrott RE, et al. A nonsense mutation in Ikbkb causes combined immunodeficiency. *Blood* 2014;124:2046-50.
42. Keller B, Cseresnyes Z, Stumpf I, Wehr C, Fliegauf M, Bulashevskaya A, et al. Disturbed canonical nuclear factor of κ light chain signaling in B cells of patients with common variable immunodeficiency. *J Allergy Clin Immunol* 2017;139:220-31.e8.
43. Chen K, Coonrod EM, Kumanovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF- κ B pathway in the pathogenesis of common variable immunodeficiency. *Am J Hum Genet* 2013;93:812-24.
44. Lee CE, Fulcher DA, Whittle B, Chand R, Fewings N, Field M, et al. Autosomal dominant B cell deficiency with alopecia due to a mutation in NFKB2 that results in nonprocessable p100. *Blood* 2014;124:2964-72.
45. Fliegauf M, Bryant VL, Frede N, Slade C, Woon ST, Lehnert K, et al. Haploinsufficiency of the NF- κ B1 subunit p50 in common variable immunodeficiency. *Am J Hum Genet* 2015;97:389-403.
46. Boztug H, Hirschmugl T, Holfer W, Lakatos K, Kager L, Trapin D, et al. NF- κ B1 haploinsufficiency causing immunodeficiency and EBV driven lymphoproliferation. *J Clin Immunol* 2016;36:533-40.
47. Sasaki Y, Imai K. Roles of the NF- κ B pathway in B lymphocytes biology. *Curr top Microbiol Immunol* 2016;393:177-209.
48. Lougaris V, Tabellini G, Vitali M, Baronio M, Patrizi O, Tampella C, et al. Defective natural killer cell cytotoxic activity in NF- κ B2 CVID like disease. *J Allergy Clin Immunol* 2015;135:1641-3.
49. Ikeda F. Linear ubiquitination in signals in adaptive immune response. *Immunol Rev* 2015;266:222-36.
50. Ombrello MJ, Kastner DL, Milner JD. HOIL and water: the two faces of HOIL-1 deficiency. *Nat Immunol* 2012;13:1133-5.
51. Boisson B, Laplantine E, Prando C, Giliani S, Israelsson E, Xu Z, et al. Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat Immunol* 2012;13:1178-86.
52. Boisson B, Laplantine E, Dobbs K, Cobat A, Tarantino N, Hazen M, et al. Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis and lymphangiectasia. *J Exp Med* 2015;212:939-51.
53. Donley DK. TORCH infections in the newborn. *Semin Neurol* 1993;13:106-15.
54. Vivarelli R, Grosso S, Cioni M, Galluzzi P, Monti L, Morgese G, et al. Pseudo-TORCH syndrome or Baraitser-Reardon syndrome: diagnostic criteria. *Brain Dev* 2001;23:18-23.
55. Rice GI, Kasher PR, Forte GM, Mannion NM, Greenwood SM, Szykiewicz M, et al. Mutations in ADAR1 cause Aicardi-Goutières syndrome associated with a type I interferon signature. *Nat Genet* 2012;44:1243-8.
56. Meuwissen ME, Schot R, Buta S, Oudesluijs G, Tinschert S, Speer SD, et al. Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo TORCH syndrome. *J Exp Med* 2016;213:1163-74.
57. Yang Z, Xian H, Hu J, Tian S, Qin Y, Tian S, et al. USP18 negatively regulates NF- κ B signaling by targeting TAK1 and NEMO for deubiquitination through distinct mechanisms. *Sci Rep* 2015;5:12738.
58. Zhang D, Zhang DE. Interferon-stimulated gene 15 and the protein ISGylation system. *J Interferon Cytokine Res* 2011;31:119-30.
59. Zhang X, Bogunovic D, Payell-Brogard B, Francois-Newton V, Speer SD, Yuan C, et al. Human intracellular ISG15 prevents interferon a/b over amplification and auto-immunity. *Nature* 2015;517:89-93.
60. Zhou Q, Wang H, Schwartz DM, Stoffels M, Park TH, Zhang Y, et al. Loss of function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early onset autoimmunity disease. *Nat Genet* 2016;48:67-73.
61. Catrysse L, Verecke L, Beyaert R, van Loo G. A20 in inflammation and autoimmunity. *Trends Immunol* 2016;35:22-31.
62. Ma A, Malynn BA. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol* 2012;12:774-85.
63. Skaug B, Chen J, Du F, He J, Ma A, Chen ZJ. Direct, noncatalytic mechanism of IKK inhibition by A20. *Mol Cell* 2011;44:559-71.
64. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, et al. Failure to regulate TNF-induced NF- κ B and cell death responses in A20-deficient mice. *Science* 2000;289:2350-4.
65. Sun SC. The noncanonical NF- κ B pathway. *Immunol Rev* 2012;246:125-40.
66. Willmann KL, Klaver S, Doğru F, Santos-Valente E, Garncarz W, Bilic I, et al. Biallelic loss-of-function mutation in NIK causes a primary immunodeficiency with multifaceted aberrant lymphoid immunity. *Nat Commun* 2014;5:5360.
67. Hahn M, Macht A, Waisman A, Hövelmeyer N. NF- κ B-inducing kinase is essential for B-cell maintenance in mice. *Eur J Immunol* 2016;46:732-41.
68. Cildir G, Low KC, Tergaonkar V. Noncanonical NF- κ B signaling in health and disease. *Trends Mol Med* 2016;22:414-29.
69. Elliott PR, Nielsen SV, Marco-Casanova P, Fill BK, Keukeotten K, Mailand N, et al. Molecular basis and regulation of Otulin- LUBAC interaction. *Mol Cell* 2014;54:335-48.
70. Damgaard RB, Walker JA, Marco-Casanova P, Morgan NV, Titheradge HL, Elliott PR, et al. The deubiquitinase OTULIN is an essential negative regulator of inflammation and autoimmunity. *Cell* 2016;166:1215-30.
71. Zhou Q, Yu X, Demirkaya E, Deutch N, Stone D, Tsai WL, et al. Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci U S A* 2016;113:10127-32.