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Genomics, Biology, and Human Illness: Advances in the Monogenic Autoinflammatory Diseases

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SYNOPSIS

The monogenic autoinflammatory diseases are a group of illnesses with prominent rheumatic manifestations that are characterized by genetically-determined recurrent sterile inflammation, and are thus inborn errors of innate immunity. Molecular targeted therapies against inflammatory cytokines such as IL-1 and TNF and intracellular cytokine signaling pathways have proven effective in many cases. Emerging next generation sequencing technologies have accelerated the identification of previously unreported genes causing autoinflammatory diseases. In this review we will cover several of the prominent recent advances in the field of autoinflammatory diseases, including gene discoveries, the elucidation of new pathogenic mechanisms, and the development of effective targeted therapies.

Keywords

haploinsufficiency of A20 (HA20); otulipenia; deficiency of ADA2 (DADA2); interferonopathy; CANDLE/PRAAS; SAVI; NLRC4 inflammasome; pyrin inflammasome

INTRODUCTION

Autoinflammatory diseases (AID) are a group of disorders characterized by seemingly unprovoked inflammation that may be recurrent or sometimes nearly continuous. The term "autoinflammatory" first appeared in the literature in 1999 to describe two monogenic disorders with recurrent fevers and episodes of systemic inflammation without high-titer autoantibodies or antigen-specific T cells: familial Mediterranean fever (FMF) and the then newly-described TNF receptor associated periodic syndrome (TRAPS)¹. At present more

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than 20 monogenic AID have been reported. The clinical manifestations of AID are typically driven by genetically-determined dysregulation of innate immunity, which results in overproduction of inflammatory cytokines, such as IL-1 β , IL-6, IL-18, TNF, and type I interferon (IFN). Specific treatments targeting these cytokine signaling pathways have been proven to be effective in many AID patients, highlighting the importance of accurate genetic diagnosis and detailed molecular pathophysiology.

In this article, we review some of the recent advances in the field of AID over the last three years, including the discovery of several newly identified monogenic disorders (Table 1). We also focus on recent insights into the pathogenesis of FMF to demonstrate how genetics and basic biology have synergized to demystify one important mechanism of host-pathogen interaction.

THE DEUBIQUITINASE DEFICIENCIES

NF- κ B denotes a group of transcription factors that regulate the expression of genes involved in the cell cycle, immune response, differentiation, and DNA repair. This signaling pathway is in part regulated by ubiquitination, a protein post-transcriptional modification process²³. The deubiquitinases (DUBs) are a group of enzymes that specifically remove ubiquitin (Ub) moieties from target proteins, and their dysregulation has been reported to result in various human diseases⁴. Several DUBs, including A20, CYLD, OTULIN, and OTUD7B (Cezanne), act as negative regulators of NF- κ B signaling². Prior to 2016, CYLD was the only DUB for which germline mutations had been implicated in a Mendelian human disease⁵.

Haploinsufficiency of A20 (HA20)

A20 is a DUB that plays a key inhibitory role in the NF- κ B proinflammatory pathway. The inhibitory function of A20 is coordinately effected by its N-terminal ovarian tumor (OTU) domain-mediated DUB activity and by its C-terminal zinc finger-mediated E3 ubiquitin ligase activity. Thus, A20 removes lysine 63 (K63)-linked Ub chains from proinflammatory signaling complexes, leading to their disassembly, and then conjugates the constituent proteins with lysine 48 (K48)-linked Ub chains, marking them for proteasomal degradation. Hence, the net effect of A20 is anti-inflammatory, and a deficiency of A20 would be predicted to cause unchecked inflammation.

In 2016, Zhou et al reported six families with dominantly inherited truncating mutations in the *TNFAIP3* gene, which encodes A20⁶. Clinical manifestations included early-onset fevers, arthralgia, oral and genital ulcers, and ocular inflammation, in some cases resembling Behçet's disease. Five of the mutations were in the OTU domain, while one was in the zinc finger domain. Mutant A20 demonstrated no inhibitory effect on the NF- κ B pathway, while a mixture of wild type and mutant A20 had substantial inhibitory activity, suggesting that the mutant proteins are likely to act through haploinsufficiency rather than a dominant-negative effect. In vitro reconstitution experiments showed accumulation of K63-Ub on RIPK1, one of the A20 substrates, an effect that was also confirmed in patients' cells. Patients' peripheral blood mononuclear cells (PBMCs) and fibroblasts also demonstrated strong phosphorylation of I κ B α , IKK α / β , and p38 with and without TNF stimulation, consistent with constitutive

NF- κ B activity. Spontaneous NLRP3 inflammasome activation leading to IL-1 β release was observed in PBMCs, and one of the patients showed a good clinical response to IL-1 β inhibition, consistent with previous reports suggesting the role of A20 as a negative regulator of the NLRP3 inflammasome in mice⁷⁸. Zhou et al dubbed this novel Mendelian disease *haploinsufficiency of A20 (HA20)* (Figure 1A).

Recent studies suggest an essential role of A20 in the development and appropriate regulation of immune cells, and its dysregulation has been linked to various human diseases. Common nucleotide variants in *TNFAIP3* have been associated with multiple autoimmune diseases, including systemic lupus erythematosus (SLE), type I diabetes, inflammatory bowel disease, ankylosing arthritis, Sjögren's syndrome, and rheumatoid arthritis⁹. Furthermore, somatic loss-of-function mutations in A20 have been described in B cell lymphoma, which suggests its role as a tumor-suppressor gene¹⁰. Complete loss of A20 in mice (*Tnfaip3*^{-/-}) resulted in early lethality due to persistent NF- κ B activation and severe multiorgan inflammation¹¹, whereas immune-cell specific ablation resulted in autoimmunity, such as SLE¹². One of the reported HA20 cases initially carried the diagnosis of SLE⁶, and recently a new case of HA20 from Japan was reported to have autoimmune lymphoproliferative syndrome (ALPS)¹³. Taken together, these reports underscore the importance of A20 in immune regulation, and suggest that further study is needed to define the full clinical spectrum of HA20.

Otulipenia/Otulin-Related Autoinflammatory Syndrome (ORAS)

Linear (or methionine 1-linked) ubiquitination is catalyzed by the linear ubiquitin assembly complex (LUBAC)¹⁴¹⁵, consisting of HOIL-1, HOIP, and SHARPIN. LUBAC plays a critical role in the activation of NF- κ B signaling by ligating linear-Ub to its target proteins, which include NEMO and RIPK1. OTULIN (FAM105B) is a DUB that, as a cysteine protease, exclusively hydrolyzes linear-Ub, prevents baseline accumulation of linear-Ub on LUBAC components, and restricts ubiquitination of LUBAC target proteins (Figure 1B)¹⁶¹⁷¹⁸. OTULIN has an N-terminal PUB-interacting site through which it interacts with HOIP¹⁹, and a C-terminal OTU domain that mediates its DUB activity.

Zhou et al and Damgaard et al independently reported that homozygous mutations of OTULIN cause a novel systemic autoinflammatory disorder²⁰²¹. These patients were characterized by neonatal-onset fever, neutrophilic dermatosis, panniculitis, lipodystrophy, joint swelling, diarrhea, and failure to thrive. Due to their dermatologic findings, two patients were initially diagnosed as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE). Two of the mutations were missense substitutions that were predicted to be damaging and the other was a frameshift mutation. Indeed, protein expression of OTULIN was severely reduced in all the patients, suggesting the instability of the mutant proteins. Zhou and colleagues further demonstrated that patients' cells showed enhanced phosphorylation of I κ B α and linear-Ub accumulation on NEMO after cytokine stimulation. Overproduction of inflammatory cytokines including TNF, IL-1 β , IL-6, IL-17, IL-18 and IFN γ was detected in patients' serum samples. Anti-TNF therapy was effective in controlling disease activity as well as suppressing inflammatory markers in the blood.

OTULIN-null mice (*gumby/gumby*) are embryonic lethal due to defects in angiogenesis¹⁸. Damgaard et al generated mice with an inducible system of immune cell-specific OTULIN ablation. Strikingly, the immune cell-specific OTULIN ablation resulted in rapid weight loss and systemic inflammation, which was completely reversed by anti-TNF treatment. These authors further tested the effects of lineage-specific OTULIN deletion in multiple cell types. Only OTULIN disruption in the myeloid cell lineage led to inflammatory phenotypes, which included enlargement of lymphoid organs, immune cell infiltration in the liver, and the elevation of serum inflammatory cytokines. T- or B- cell specific OTULIN ablation did not result in an overt phenotype. However, HOIP and SHARPIN expression levels were strongly reduced, presumably due to protein destabilization, which suggests a possible unexpected mechanism of lineage-specific LUBAC regulation.

While loss of OTULIN leads to excessive inflammation through accumulation of linear-Ub, germline deficiencies of LUBAC also result in autoinflammation. Boisson et al reported three *RBCK1* (HOIL-1) deficient and one *RNF31* (HOIP) deficient patients, characterized by immunodeficiency, rash, gastrointestinal manifestations, myopathy, and systemic autoinflammation^{22,23}. The patients' fibroblasts showed less linear-Ub accumulation and reduced NF- κ B signaling, relative to controls, while patients' monocytes were hyperactive upon IL-1 β stimulation. Furthermore, loss of SHARPIN in mice is responsible for the paradoxical phenotypes of the chronic proliferative dermatitis mouse (cpdm), in which systemic multiorgan inflammation coexists with immunodeficiency^{24,25,26}. These reports underscore the importance of linear ubiquitination in both pro- and anti-inflammatory pathways.

DEFICIENCY OF ADA2 (DADA2)

In 2014 Zhou et al²⁷ and Navon-Elkan et al²⁸ identified biallelic loss-of-function *CECR1* mutations in patients presenting with fevers and early-onset strokes and/or with vasculitis resembling polyarteritis nodosa (PAN), a systemic necrotizing vasculitis typically affecting medium-sized muscular arteries²⁹. Zhou and her colleagues reported nine patients with fevers, early-onset (< 5 years old) lacunar strokes, livedoid rash, hepatosplenomegaly, cytopenia, and systemic vasculopathy, including two patients with PAN and one with small-vessel vasculitis. Eight of the 9 patients had histories of lacunar strokes mainly affecting the deep-brain nuclei and the brain stem. Several strokes were hemorrhagic, or underwent hemorrhagic transformation, leading to long-term disability. Most of the strokes occurred during episodes of systemic inflammation. These patients also presented with various sequelae of systemic vascular disease, including livedo racemosa, myositis, portal hypertension, and ophthalmologic complications. Indeed, biopsy samples from skin, liver, and brain exhibited vasculopathic changes, including impaired endothelial integrity, endothelial cellular activation, and inflammation. Four patients had hypogammaglobulinemia, and two of them had multiple episodes of bacterial and viral infections.

Simultaneously and independently, Navon Elkan and her colleagues reported 24 patients with PAN with biallelic *CECR1* mutations, 19 of whom were of Georgian Jewish ancestry. Among them, 18 presented with childhood-onset PAN (<10 years old), including 6 who

received the diagnosis during infancy (< 1 year old). Most of the patients had cutaneous involvement, most commonly livedo racemosa. Five patients had episodes of either strokes or intracranial hemorrhage, whereas 10 had signs of peripheral neuropathy. Aneurysm formation in visceral arteries was observed in 6 patients, with associated renal hypertension and gastrointestinal manifestations.

CECR1 encodes adenosine deaminase 2 (ADA2), which can convert adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine³⁰. Although ADA2 has partial structural homology with ADA (ADA1), the deficiency of which causes human severe combined immunodeficiency (SCID) through the intracellular accumulation of toxic nucleotides, these two enzymes differ in many aspects³⁰. Whereas ADA1 acts as a monomer and is primarily localized intracellularly, ADA2 acts as a dimer and is secreted into the extracellular space. The patients described in both reports had a marked reduction of ADA2 protein concentrations and ADA2-specific enzymatic activity in the blood, suggesting that the detected mutations were loss-of-function. In the structural analysis, the missense mutations were predicted to affect the catalytic and dimerization domains or protein stability. Knock-down of *cecr1b* in zebrafish embryos resulted in intracranial hemorrhages and neutropenia, which were rescued by co-injection with human wild-type *CECR1* (but not mutant *CECR1*), establishing the pathogenicity of the mutations. Zhou and her colleagues therefore proposed the term *deficiency of adenosine deaminase 2 (DADA2)* to denote the human disease.

ADA2 is expressed in the myeloid lineage and, once secreted, it induces differentiation of monocytes into macrophages, possibly by binding proteoglycan-like structures on the cellular surface³¹. Indeed, DADA2 patient monocytes showed impaired differentiation toward the anti-inflammatory (M2) macrophage population under standard culture conditions, thus leading to polarized differentiation toward the pro-inflammatory (M1) macrophage subset²⁷. A recent transcriptome-wide analysis utilizing DADA2 patients' blood samples displayed a strong upregulation of neutrophil-related genes as well as a moderate interferon signature, and the authors also reported the accumulation of myeloperoxidase in patients' polymorphonuclear cells³². Zhou et al further reported that the DADA2 patients' brain and skin samples showed substantial endothelial activation and damage, and upregulation of inflammatory cytokines²⁷. Co-culture of patients' monocytes with a human primary endothelial cell layer led to considerable disruption of its integrity. These studies suggest that the deficiency of ADA2 results in vascular damage at least in part mediated by skewed monocytic differentiation and neutrophil activation.

It is noteworthy that all of the Georgian Jewish patients in the report from Navon Elkan were homozygous for a mutation encoding a p.Gly47Arg substitution. The carrier frequency of this mutation in the endogamous Georgian Jewish population was 0.102, which is consistent with the apparently high prevalence of this disease in this population. Conserved haplotypes were detected around several missense mutations including p.Gly47Arg, suggesting the existence of a possible founder effect²⁷. Interestingly, a heterozygous p.Tyr453Cys *CECR1* mutation was identified in two siblings with late-onset lacunar strokes in the Siblings with Ischemic Stroke Study (SWISS)^{33,27}. Although no GWAS loci for stroke have been identified in the *CECR1* gene region, the effect of this gene on non-Mendelian cases of stroke and other vascular diseases should be further pursued.

Recent reports have broadened the clinical spectrum of DADA2 beyond the typical clinical picture of systemic inflammation presented in the first papers. *CECR1* mutations have been demonstrated in patients with autoimmunity, lymphoproliferation, and a combined immunodeficiency (CID)³⁴, as well as patients presenting primarily with common variable immunodeficiency (CVID)³⁵. The lymphoproliferative picture is shared by a mutation-positive patient who was diagnosed with Castleman's disease and responded to anti IL-6 treatment³⁶. Biallelic *CECR1* mutations have also been found in patients presenting with anemia, thrombocytopenia, and splenomegaly, leading to the initial clinical diagnosis of Diamond-Blackfan anemia or storage disease³⁷. Recently a biallelic 770-kb deletion of chromosome 22q11.1 encompassing both *CECR1* and *IL17RA* (encoding the IL-17 receptor A) was reported in two siblings with a history of both mucocutaneous infection and early-onset systemic vasculitis³⁸. This finding, taken together with the 28-kb deletion in the *CECR1* locus reported by Zhou et al²⁷, indicates the importance of structural genomic analysis in the genetic diagnosis of DADA2.

Given the specter of stroke, vasculitis, and the other possible manifestations of DADA2, treatment strategies have been the subject of intense investigation. In many cases, corticosteroids, methotrexate, cyclophosphamide, azathioprine, and IL-1 inhibitors have been ineffective. Navon Elkan et al reported the use of anti-TNF agents in ten DADA2 vasculitis patients, among which eight demonstrated a complete response²⁸. This strategy has been further supported by a recent report from Ombrello and colleagues, demonstrating that anti-TNF treatment has completely prevented the recurrence of strokes in 15 DADA2 patients with a previous history of stroke³⁹. Hematopoietic stem cell transplantation (HSCT) is another possible therapeutic strategy, because the pathogenesis of DADA2 appears to derive mainly from the myeloid cell lineage. Indeed, the effectiveness of HSCT was initially reported by Van Eyck et al⁴⁰, followed by a number of papers^{41,37}, in which HSCT normalized the plasma level of ADA2 and suppressed disease manifestations. It will be important to establish the risk-benefit ratios for anti-TNF, HSCT, and other potential therapies in various clinical settings across the widening spectrum of DADA2.

Lastly, although the zebrafish data of Zhou et al clearly establish the importance of *CECR1* in vascular and myeloid development, the field has been hampered by the lack of an obvious murine *CECR1* orthologue that would permit the development of a mouse model. Whether through the development of alternative animal models or through the more detailed study of leukocyte and endothelial biology in DADA2 patients, there is still much to be learned about the basic biology of this disease.

TYPE I INTERFERONOPATHIES

Type I interferons (mainly IFN- α and - β) are a group of cytokines that play an important role in host defense. Binding of type I IFN to the IFN- α/β receptor (IFNAR) induces the transcription of hundreds of genes called IFN-stimulated genes (ISGs) by activating the JAK-STAT signaling pathway. The overproduction of type I IFN leads to a group of Mendelian inherited diseases called type I interferonopathies. For example, Aicardi-Goutières disease derives from either loss-of-function mutations in cytoplasmic nucleic acid metabolism or gain-of-function mutations in pattern recognition receptors such as MDA5

(encoded by the *IFIH1* gene), leading to severe encephalopathy and systemic autoimmunity resembling SLE (see article by Hiraki and Silverman in this issue for further discussion of Aicardi- Goutières disease). Upregulation of ISGs in the peripheral blood ("IFN-signature") was proposed as a screening method for interferonopathy, analogous to the upregulation of type I IFN signaling in SLE patients⁴²⁴³⁴⁴.

STING-Associated Vasculopathy with Onset in Infancy (SAVI)

Recently the cGAS-cGAMP-STING pathway was identified as the mechanism for the detection of cytoplasmic double-stranded DNA (dsDNA)⁴⁵. Upon recognition and binding of cytoplasmic dsDNA, cyclic GMP-AMP synthase (cGAS) synthesizes cyclic GMP-AMP (cGAMP) as a second messenger, which then activates the stimulator of interferon genes (STING), an endoplasmic reticulum (ER)-resident adapter molecule. cGAMP induces the translocation of STING from the ER to the ER-Golgi intermediate compartment (ERGIC) to recruit TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), resulting in upregulation of type I IFN transcription (Figure 2).

In 2014 Liu et al identified de novo germline or somatic mosaic gain-of-function mutations in *TMEM173*, encoding STING, in six patients with early-onset autoinflammatory disorders manifesting dermatologic and pulmonary involvement⁴⁶. Skin lesions were seen in all six patients, with telangiectatic, pustular, or blistering rashes and gangrenous lesions, the latter of which were exacerbated by cold exposure and even required surgical amputations. Microscopically these skin lesions were characterized by marked vascular inflammation around capillaries with fibrin and immune complex deposition. Concomitantly various degrees of interstitial lung disease were observed in five of six patients. Lung biopsies showed a scattered lymphocytic inflammatory infiltrate, interstitial fibrosis, and emphysematous changes. Strong IFN signatures as well as constitutive phosphorylation of STAT1 in patients' lymphocytes were observed, consistent with the constitutive activity of mutant STING in an in vitro assay. Stimulation of patients' fibroblasts with cGAMP induced further *IFNB1* upregulation. Patients' vascular endothelial cell layers from lesional skin samples expressed markers of endothelial inflammation and activation. These authors proposed the term STING-associated vasculopathy with onset in infancy (SAVI) to denote this condition.

To date, more than 20 patients from multiple pedigrees have been reported with gain-of-function mutations in *TMEM173*^{46,4748495051525354}. Most of the mutations encode amino acid substitutions located close to the dimerization site of STING, and in fact two recombinant mutants (p.N154S and p.V155M) were reported to form a stable homodimer⁴⁶. Dobbs et al recently demonstrated that disease-associated STING mutants trafficked to the ERGIC without cGAMP stimulation, and also that these active mutants were less susceptible to degradation, both of which could explain the constitutively active characteristics of STING mutants in SAVI⁵⁵. Melki et al identified three novel missense mutations in STING (p.C206Y, p.R281Q, and p.R284G), all of which lie outside the linker region important for homo-dimerization⁵⁴. The precise molecular mechanism of STING mutants in SAVI remains to be defined.

While therapies such as corticosteroids and DMARDs, or various inhibitors of TNF, IL-1, and IL-6, have been reported to elicit minimal clinical responses, accumulating evidence suggests the effectiveness of JAK inhibition in SAVI. Ex vivo treatment with any of three JAK inhibitors blocked the constitutive phosphorylation of STAT1 in a patient's B and CD4+ T cells⁴⁶. Recently two papers reported the clinical efficacy of JAK inhibitors in SAVI^{52,56}, with reduction in febrile episodes and almost complete resolution of dermatologic lesions. Early genetic diagnosis and treatment may prevent the eventual need for surgical amputations and the irreversible pulmonary damage of SAVI.

Chronic Atypical Neutrophilic Dermatositis with Lipodystrophy and Elevated Temperature (CANDLE)/Proteasome-Associated Autoinflammatory Syndrome (PRAAS)

Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE), characterized by fever, panniculitis, and brain calcification, is another form of type I interferonopathy with biallelic loss-of-function mutations in *PSMB8*, which encodes the inducible proteasome component $\beta 5i$ ^{57,58,59,60}. It is one of several clinical conditions that collectively are denoted the proteasome-associated autoinflammatory syndrome (PRAAS). Although a strong IFN signature in CANDLE patients suggested the association between proteasome dysfunction and IFN signaling, the direct molecular mechanism had not been well defined. Brehm et al reported eight patients with mutations in four proteasome genes, *PSMA3* (encoding the $\alpha 7$ subunit), *PSMB4* (encoding $\beta 7$), *PSMB9* (encoding $\beta 1i$), and *POMP* (encoding the proteasome maturation protein), and also one novel mutation in *PSMB8*⁶¹. Of note, six of the reported patients were compound heterozygous for two different genes described above, which suggested a "digenic inheritance" model in CANDLE. In addition, one patient was heterozygous for the *POMP* frameshift mutation, which likely causes haploinsufficiency, suggesting a novel autosomal-dominant mode of inheritance. The authors also demonstrated that proteasome inhibition in fibroblasts from healthy individuals, either by knockdown or pharmacological inhibition, resulted in strong upregulation of IFN related genes, which recapitulated the patients' IFN signature. A clinical trial of a JAK inhibitor for CANDLE is ongoing (NCT01724580), with promising preliminary results⁶².

NLRC4-RELATED AUTOINFLAMMATORY SYNDROMES

NLRC4, a member of the nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) family, assembles with NAIP and ASC to form an inflammasome complex upon the intracellular entry of ligands such as bacterial flagellin or components of the bacterial type 3 secretion system. As a result, the NLRC4 inflammasome mediates the autocatalysis of procaspase-1 to the enzymatically active caspase-1, which subsequently catalyzes the cleavage of pro-IL-1 β and pro-IL-18 to their biologically active mature forms, and also induces a rapid lytic cell death called pyroptosis.

In 2014 Canna et al and Romberg et al independently reported that heterozygous gain-of-function mutations of *NLRC4* (p.T337S and p.V341A, respectively) caused an autoinflammatory disease. The phenotypes of these patients were characterized by early-onset multiple fever episodes, enterocolitis, splenomegaly, macrophage activation syndrome

(MAS)-like flares, and persistent serum IL-18 elevation^{63,64}. In both reports, in vitro overexpression of mutant NLRC4 protein led to increased cleavage of caspase-1, and patients' macrophages exhibited increased production of IL-1 β and IL-18, more frequent formation of ASC specks, and increased susceptibility to cell death, suggesting that these mutations result in constitutive activation of the NLRC4 inflammasome.

Concurrently, Kitamura et al reported that another mutation in *NLRC4* (p.H443P) caused a somewhat milder phenotype with cold-induced fever and urticaria, which resembled familial cold autoinflammatory syndrome (FCAS)⁶⁵. Transgenic mice expressing this mutant *NLRC4* allele developed severe inflammatory infiltrates in the skin and joints, erosion of bones, splenomegaly, and elevated levels of IL-1 β , IL-17A, and G-CSF in the blood. Hypercytokinemia could be induced by adoptive transfer of bone marrow from mutant mice into wild type recipients. Cold exposure of mutant mice augmented the cutaneous inflammation, which recapitulated the patients' phenotype.

Recently, a fourth mutation of *NLRC4* (p.T177A) was reported in a patient with periodic fever, rash, arthralgia, sensorineural hearing loss, chronic meningitis, and cerebral atrophy, all of which are consistent with neonatal onset multisystem inflammatory disease (NOMID)⁶⁶. In this paper, multiple iPSC-derived monocytic lineage cells from the patient showed a differential pattern of inflammasome activation, and whole exome sequencing between these normal and inflammatory groups of iPSCs identified the *NLRC4* p.T177A mutation, suggesting that the patient had somatic mosaicism of this *NLRC4* mutation.

A structural study showed that ADP-bound NLRC4 is in an autoinhibited state that is achieved by an ADP-mediated intramolecular interaction⁶⁷. Disruption of this interaction by ligand binding may facilitate conformational changes and attenuate ADP binding, both of which could result in NLRC4 inflammasome activation. It is noteworthy that all of the mutations identified in human *NLRC4*-related autoinflammatory syndromes are in close proximity to the ADP-binding site and that p.Thr177 and p.His443 individually form a hydrogen bond to the ADP molecule. This clustering of disease-causing mutations in the nucleotide-binding domain (NBD), as well as the phenotypic variability and the presence of somatic mosaicism discussed above, all suggest the similarities between *NLRC4*-related autoinflammatory syndromes and cryopyrin-associated periodic syndrome (CAPS) caused by *NLRP3* mutations.

Hemophagocytic disorders such as MAS and hemophagocytic lymphohistiocytosis (HLH) are life-threatening sepsis-like conditions notable for acute cytopenias, hyperferritinemia, coagulopathy, and multiple organ damage, and if not promptly treated, they are associated with high mortality rates. In addition to *NLRC4*-MAS, several conditions such as familial HLH and systemic onset juvenile idiopathic arthritis (sJIA) are predisposed to MAS. During the flares, serum total IL-18 as well as free IL-18 are extraordinary high, although its role in the pathogenesis of MAS/HLH remains controversial⁶⁸. Recently administration of recombinant human IL-18 binding protein successfully rescued a patient with *NLRC4*-MAS, who did not respond to a combination of immunosuppressive drugs, anti-IL-1, anti-TNF, and $\alpha_4\beta_7$ integrin inhibitory therapies⁶⁹. This not only substantiates a proinflammatory role for

IL-18 in MAS, but also raises a possible new treatment option for various MAS/HLH-inducing clinical conditions.

RECENT ADVANCES IN FMF AND THE PYRIN INFLAMMASOME

FMF is the longest-recognized and one of the most extensively studied hereditary autoinflammatory disorders. It is characterized by recurrent 1–3 day febrile attacks accompanied by serositis, synovitis, and/or cutaneous inflammation. Two independent consortia identified *MEFV*, the gene mutated in FMF, by positional cloning in 1997^{70,71}. Since that time, the mechanism by which mutations in pyrin, the encoded protein, cause FMF has been the topic of intense investigation. Although at first FMF was hypothesized to be an autosomal recessive disease deriving from biallelic loss-of-function mutations mainly in exon 10 of *MEFV* (encoding the B30.2 domain of pyrin), subsequent reports of 30–40% of FMF patients carrying only one mutated allele of *MEFV*, coupled with the conspicuous absence of null mutations, raised the possibility that FMF is caused by gain-of-function mutations in *MEFV*.

Human pyrin consists of an N-terminal PYRIN (PYD) domain, a B-box zinc-finger domain, a coiled-coil domain, and a C-terminal ~200 amino acid B30.2 domain (also known as the rfp/PRY/SPRY domain). As noted above, the majority of FMF-causing mutations are located in the B30.2 domain⁷². The PYD domain interacts with an adapter protein, apoptosis-associated speck-like protein with a caspase recruitment domain (ASC), through a homotypic PYD-PYD interaction⁷³. Chae et al reported in 2011 that homozygous knockin mice harboring any one of three human B30.2 domains with an FMF mutation showed constitutive activation of caspase-1 in macrophages and IL-1 β secretion after lipopolysaccharide (LPS) stimulation (without a second signal), which was completely dependent on ASC and caspase-1 but not on NLRP3⁷⁴. This paper also demonstrated that this prominent inflammatory phenotype was not induced by knockout of the murine *Mefv* gene, and crosses between knockin and knockout mice supported the hypothesis that FMF-associated mutations are gain-of-function with a gene-dosage effect.

In 2014 Xu et al published a breakthrough paper that shed considerable light on the mechanism by which pyrin contributes to host defense⁷⁵. These authors demonstrated that pyrin inflammasome activation is induced by modifications of host RhoA by certain bacterial toxins. RhoA inactivation is a shared mechanism of bacterial virulence that prevents the reorganization of the host actin cytoskeleton and thus inhibits leukocyte migration, phagocytosis, and degranulation. These pyrin inflammasome-activating modifications of RhoA include monoglucosylation, adenylation, ADP-ribosylation, and deamidation, occurring on different residues. Thus, it was hypothesized that pyrin would recognize these RhoA modifications by sensing a downstream effect, although the molecular details were at the time unknown.

In 2016 Park and colleagues elucidated the molecular mechanism by which RhoA inactivation leads to pyrin inflammasome activation (Figure 3)⁷⁶. Under physiological conditions RhoA induces the phosphorylation of pyrin on its residues p.Ser208 and p.Ser242 through two effector kinases, PKN1 and PKN2. Pyrin phosphorylation on these residues

permits its interaction with 14-3-3, a regulatory protein, that in turn inhibits pyrin inflammasome formation. Of note, the binding of 14-3-3 and PKN1/2 to FMF-associated mutant pyrin is substantially reduced, and the constitutive activation of the pyrin inflammasome in FMF knockin mice as well as FMF patient cells are inhibited by pharmacological PKN activation. Bacterial inactivation of RhoA diminishes PKN activity and thus favors the dephosphorylated form of pyrin that can assemble an inflammasome. This paradigm, sometimes termed the ‘guard mechanism’⁷⁶, by which microbial products activate innate immunity through their indirect biologic effects on the cell, is common among plants, but the pyrin inflammasome represents the first documented example of the guard mechanism in the animal kingdom.

The importance of pyrin p.Ser242 phosphorylation was further highlighted by the recent identification of a novel pyrin-related disorder distinct from FMF, called pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND). In 2016 Masters et al reported the heterozygous p.Ser242Arg mutation in a group of patients with neutrophilic dermatosis (severe acne, sterile skin abscess, and pyoderma gangrenosum), recurrent long-lasting fever episodes, arthralgia, and myalgia/myositis, associated with the upregulation of systemic inflammatory markers⁷⁷. The prominent dermatologic manifestations of these patients resemble the cutaneous features of pyogenic arthritis with pyoderma gangrenosum and acne (PAPA) syndrome, which is caused by mutations in *PSTPIP1*, an interactor of pyrin. The p.Ser242Arg mutation of pyrin dramatically reduced its affinity to 14-3-3, and resulted in constitutive activation of the pyrin inflammasome.

Quite unexpectedly, Park's report above also demonstrated a role for the pyrin inflammasome in the pathophysiology of another AID, mevalonate kinase deficiency (also known as hyper-IgD syndrome, or HIDS). HIDS is caused by recessive loss-of-function mutations in *MVK*, encoding mevalonate kinase, an enzyme that is important both in cholesterol biosynthesis and in the synthesis of nonsterol isoprenes, such as geranylgeranyl pyrophosphate. Pharmacological inhibition of cholesterol synthesis by statins, which mimics the molecular pathogenesis of HIDS, had been shown to induce IL-1 β release, but the molecular mechanism was unknown⁷⁸⁷⁹. Reasoning that geranylgeranylation of RhoA is important for its trafficking to the plasma membrane⁸⁰, and that the impairment of RhoA geranylgeranylation would inhibit its activity and thus result in pyrin inflammasome activation, Park et al showed that statin treatment dissociates RhoA from the plasma membrane to the cytosol, resulting in IL-1 β production. This IL-1 β production is blocked by supplementation of geranylgeranyl pyrophosphate or pharmacological activation of PKNs, is dependent only on the pyrin inflammasome (but not on NLRP3, NLRC4, or AIM2), and is associated with decreased binding of pyrin to 14-3-3 proteins.

Finally, these recent genetic and functional data further clarify the pharmacological mechanism of colchicine, a medication used for rheumatic diseases (such as Behçet's disease) and is also one of the most commonly used and effective treatments for FMF⁸¹⁸². Colchicine is known to enhance the activity of RhoA by depolymerizing microtubules and thus releasing guanine nucleotide exchange factor (GEF)-H1, an RhoA activator⁸³. Park et al demonstrated⁷⁶ that colchicine reverses the effect of RhoA inhibition by C3 toxin from *Clostridium botulinum*, and furthermore, suppresses the spontaneous activation of the pyrin

inflammasome in FMF knockin mice and in FMF patients' cells, but not in the cells from CAPS patients. In addition, Feng Shao and colleagues⁸⁴ demonstrated that multiple microtubule-targeting drugs including colchicine, vinblastine, and paclitaxel inhibit pyrin inflammasome-mediated ASC aggregation. These data are consistent with the clinical response of FMF patients to colchicine treatment and the exquisite specificity of colchicine for FMF among the hereditary periodic fever syndromes.

SUMMARY

Recent genomic technologies including next generation sequencing (NGS) have substantially contributed to the advancement of the field of AID by catalyzing the discovery of new causative genes and pathways in AID patients. Some of these patients did not present with classical symptoms of AID, underscoring the heterogeneity of the clinical manifestations of AID. Also, the wide spectrum of clinical symptoms deriving from mutations in a single gene as exemplified by DADA2 highlights the importance of further clinical studies and the elucidation of pathogenic mechanisms. Conversely, the broad range of effects of A20 mutations from low to high penetrance and even somatic changes exemplifies the importance of comprehensive genetic approaches. The success of novel targeted therapies for previously uncontrollable AID patients emphasizes the importance of promoting translational research in this field. Exciting challenges remain to deliver rapid and accurate genetic diagnosis and effective treatments to currently undiagnosed AID patients.

References

1. McDermott MF, Aksentijevich I, Galon J, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell*. 1999; 97(1):133–144. [PubMed: 10199409]
2. Zinngrebe J, Montinaro A, Peltzer N, Walczak H. Ubiquitin in the immune system. *EMBO Rep*. 2014; 15(1):28–45. [PubMed: 24375678]
3. Panday A, Inda ME, Bagam P, Sahoo MK, Osorio D, Batra S. Transcription Factor NF-kappaB: An Update on Intervention Strategies. *Arch Immunol Ther Exp (Warsz)*. 2016; 64(6):463–483. [PubMed: 27236331]
4. Hanpude P, Bhattacharya S, Dey AK, Maiti TK. Deubiquitinating enzymes in cellular signaling and disease regulation. *IUBMB Life*. 2015; 67(7):544–555. [PubMed: 26178252]
5. Rajan N, Ashworth A. Inherited cylindromas: lessons from a rare tumour. *Lancet Oncol*. 2015; 16(9):e460–469. [PubMed: 26370355]
6. Zhou Q, Wang H, Schwartz DM, et al. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. *Nat Genet*. 2016; 48(1):67–73. [PubMed: 26642243]
7. Vande Walle L, Van Opdenbosch N, Jacques P, et al. Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. *Nature*. 2014; 512(7512):69–73. [PubMed: 25043000]
8. Duong BH, Onizawa M, Osés-Prieto JA, et al. A20 restricts ubiquitination of pro-interleukin-1beta protein complexes and suppresses NLRP3 inflammasome activity. *Immunity*. 2015; 42(1):55–67. [PubMed: 25607459]
9. Zhang M, Peng LL, Wang Y, et al. Roles of A20 in autoimmune diseases. *Immunol Res*. 2016; 64(2):337–344. [PubMed: 26135958]
10. Kato M, Sanada M, Kato I, et al. Frequent inactivation of A20 in B-cell lymphomas. *Nature*. 2009; 459(7247):712–716. [PubMed: 19412163]

11. Lee EG, Boone DL, Chai S, et al. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science*. 2000; 289(5488):2350–2354. [PubMed: 11009421]
12. Ma A, Malynn BA. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol*. 2012; 12(11):774–785. [PubMed: 23059429]
13. Takagi M, Ogata S, Ueno H, et al. Haploinsufficiency of TNFAIP3 (A20) by germline mutation is involved in autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol*. 2016
14. Sasaki K, Iwai K. Roles of linear ubiquitinylation, a crucial regulator of NF-kappaB and cell death, in the immune system. *Immunol Rev*. 2015; 266(1):175–189. [PubMed: 26085215]
15. Kirisako T, Kamei K, Murata S, et al. A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J*. 2006; 25(20):4877–4887. [PubMed: 17006537]
16. Keusekotten K, Elliott PR, Glockner L, et al. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell*. 2013; 153(6):1312–1326. [PubMed: 23746843]
17. Fill BK, Damgaard RB, Wagner SA, et al. OTULIN restricts Met1-linked ubiquitination to control innate immune signaling. *Mol Cell*. 2013; 50(6):818–830. [PubMed: 23806334]
18. Rivkin E, Almeida SM, Ceccarelli DF, et al. The linear ubiquitin-specific deubiquitinase gumbly regulates angiogenesis. *Nature*. 2013; 498(7454):318–324. [PubMed: 23708998]
19. Schaeffer V, Akutsu M, Olma MH, Gomes LC, Kawasaki M, Dikic I. Binding of OTULIN to the PUB domain of HOIP controls NF-kappaB signaling. *Mol Cell*. 2014; 54(3):349–361. [PubMed: 24726327]
20. Zhou Q, Yu X, Demirkaya E, 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(36):10127–10132. [PubMed: 27559085]
21. Damgaard RB, Walker JA, Marco-Casanova P, et al. The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell*. 2016; 166(5):1215–1230. e1220. [PubMed: 27523608]
22. Boisson B, Laplantine E, Prando C, et al. Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat Immunol*. 2012; 13(12):1178–1186. [PubMed: 23104095]
23. Boisson B, Laplantine E, Dobbs K, et al. Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. *J Exp Med*. 2015; 212(6):939–951. [PubMed: 26008899]
24. Tokunaga F, Nakagawa T, Nakahara M, et al. SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. *Nature*. 2011; 471(7340):633–636. [PubMed: 21455180]
25. Ikeda F, Deribe YL, Skanland SS, et al. SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature*. 2011; 471(7340):637–641. [PubMed: 21455181]
26. Gerlach B, Cordier SM, Schmukle AC, et al. Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature*. 2011; 471(7340):591–596. [PubMed: 21455173]
27. Zhou Q, Yang D, Ombrello AK, et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. *N Engl J Med*. 2014; 370(10):911–920. [PubMed: 24552284]
28. Navon Elkann P, Pierce SB, Segel R, et al. Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy. *N Engl J Med*. 2014; 370(10):921–931. [PubMed: 24552285]
29. Forbess L, Bannykh S. Polyarteritis nodosa. *Rheum Dis Clin North Am*. 2015; 41(1):33–46. vii. [PubMed: 25399938]
30. Zavialov AV, Engstrom A. Human ADA2 belongs to a new family of growth factors with adenosine deaminase activity. *Biochem J*. 2005; 391(Pt 1):51–57. [PubMed: 15926889]
31. Zavialov AV, Yu X, Spillmann D, Lauvau G, Zavialov AV. Structural basis for the growth factor activity of human adenosine deaminase ADA2. *J Biol Chem*. 2010; 285(16):12367–12377. [PubMed: 20147294]
32. Belot A, Wassmer E, Twilt M, et al. Mutations in CECR1 associated with a neutrophil signature in peripheral blood. *Pediatr Rheumatol Online J*. 2014; 12:44. [PubMed: 25278816]

33. Meschia JF, Brown RD Jr, Brott TG, Chukwudelunzu FE, Hardy J, Rich SS. The Siblings With Ischemic Stroke Study (SWISS) protocol. *BMC Med Genet.* 2002; 3:1. [PubMed: 11882254]
34. Van Eyck L Jr, Hershfield MS, Pombal D, et al. Hematopoietic stem cell transplantation rescues the immunologic phenotype and prevents vasculopathy in patients with adenosine deaminase 2 deficiency. *J Allergy Clin Immunol.* 2015; 135(1):283–287. e285. [PubMed: 25457153]
35. Schepp J, Bulashevskaya A, Mannhardt-Laakmann W, et al. Deficiency of Adenosine Deaminase 2 Causes Antibody Deficiency. *J Clin Immunol.* 2016; 36(3):179–186. [PubMed: 26922074]
36. Van Eyck L, Liston A, Wouters C. Mutant ADA2 in vasculopathies. *N Engl J Med.* 2014; 371(5):480.
37. Van Montfrans JM, Hartman EA, Braun KP, et al. Phenotypic variability in patients with ADA2 deficiency due to identical homozygous R169Q mutations. *Rheumatology (Oxford).* 2016; 55(5):902–910. [PubMed: 26867732]
38. Fellmann F, Angelini F, Wassenberg J, et al. IL-17 receptor A and adenosine deaminase 2 deficiency in siblings with recurrent infections and chronic inflammation. *J Allergy Clin Immunol.* 2016; 137(4):1189–1196. e1181–1182. [PubMed: 26607704]
39. Amanda, K., Ombrello, KB., Hoffmann, Patrycja, Toro, Camilo, Stone, Deborah L., Pinto-Patarroyo, Gineth, Jones, Anne, Romeo, Tina, Soldatos, Ariane, Zhou, Qing, Deutch, Natalie, Qin, Jing, Aksentijevich, Ivona, Kastner, Daniel L. The Deficiency of Adenosine Deaminase Type 2 (DADA2)-Results of Anti-TNF Treatment in a Cohort of Patients with a History of Stroke. Paper presented at: 2016 ACR/ARHP Annual Meeting; September 28, 2016, 2016; Washington DC.
40. Van Eyck L, Liston A, Meyts I. Mutant ADA2 in vasculopathies. *N Engl J Med.* 2014; 371(5):478–479.
41. van Montfrans J, Zavialov A, Zhou Q. Mutant ADA2 in vasculopathies. *N Engl J Med.* 2014; 371(5):478.
42. Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis Rheum.* 1982; 25(4):401–406. [PubMed: 6176248]
43. Gota C, Calabrese L. Induction of clinical autoimmune disease by therapeutic interferon-alpha. *Autoimmunity.* 2003; 36(8):511–518. [PubMed: 14984028]
44. Bennett L, Palucka AK, Arce E, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med.* 2003; 197(6):711–723. [PubMed: 12642603]
45. Tao J, Zhou X, Jiang Z. cGAS-cGAMP-STING: The three musketeers of cytosolic DNA sensing and signaling. *IUBMB Life.* 2016; 68(11):858–870. [PubMed: 27706894]
46. Liu Y, Jesus AA, Marrero B, et al. Activated STING in a vascular and pulmonary syndrome. *N Engl J Med.* 2014; 371(6):507–518. [PubMed: 25029335]
47. Jeremiah N, Neven B, Gentili M, et al. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. *J Clin Invest.* 2014; 124(12):5516–5520. [PubMed: 25401470]
48. Munoz J, Rodiere M, Jeremiah N, et al. Stimulator of Interferon Genes-Associated Vasculopathy With Onset in Infancy: A Mimic of Childhood Granulomatosis With Polyangiitis. *JAMA Dermatol.* 2015; 151(8):872–877. [PubMed: 25992765]
49. Omoyinmi E, Melo Gomes S, Nanthapaisal S, et al. Stimulator of interferon genes-associated vasculitis of infancy. *Arthritis Rheumatol.* 2015; 67(3):808. [PubMed: 25510345]
50. Picard C, Thouvenin G, Kannengiesser C, et al. Severe Pulmonary Fibrosis as the First Manifestation of Interferonopathy (TMEM173 Mutation). *Chest.* 2016; 150(3):e65–71. [PubMed: 27613991]
51. Konig N, Fiehn C, Wolf C, et al. Familial chilblain lupus due to a gain-of-function mutation in STING. *Ann Rheum Dis.* 2017; 76(2):468–472. [PubMed: 27566796]
52. Fremont ML, Rodero MP, Jeremiah N, et al. Efficacy of the Janus kinase 1/2 inhibitor ruxolitinib in the treatment of vasculopathy associated with TMEM173-activating mutations in 3 children. *J Allergy Clin Immunol.* 2016; 138(6):1752–1755. [PubMed: 27554814]
53. Clarke SL, Pellowe EJ, de Jesus AA, Goldbach-Mansky R, Hilliard TN, Ramanan AV. Interstitial Lung Disease Caused by STING-associated Vasculopathy with Onset in Infancy. *Am J Respir Crit Care Med.* 2016; 194(5):639–642. [PubMed: 27585386]

54. Melki I, Rose Y, Uggenti C, et al. Disease-associated mutations identify a novel region in human STING necessary for the control of type I interferon signaling. *J Allergy Clin Immunol.* 2017
55. Dobbs N, Burnaevskiy N, Chen D, Gonugunta VK, Alto NM, Yan N. STING Activation by Translocation from the ER Is Associated with Infection and Autoinflammatory Disease. *Cell Host Microbe.* 2015; 18(2):157–168. [PubMed: 26235147]
56. Seo J, Kang JA, Suh DI, et al. Tofacitinib relieves symptoms of stimulator of interferon genes (STING)-associated vasculopathy with onset in infancy caused by 2 de novo variants in TMEM173. *J Allergy Clin Immunol.* 2016
57. Liu Y, Ramot Y, Torrelo A, et al. Mutations in proteasome subunit beta type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. *Arthritis Rheum.* 2012; 64(3):895–907. [PubMed: 21953331]
58. Kitamura A, Maekawa Y, Uehara H, et al. A mutation in the immunoproteasome subunit PSMB8 causes autoinflammation and lipodystrophy in humans. *J Clin Invest.* 2011; 121(10):4150–4160. [PubMed: 21881205]
59. Arima K, Kinoshita A, Mishima H, et al. Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. *Proc Natl Acad Sci U S A.* 2011; 108(36):14914–14919. [PubMed: 21852578]
60. Agarwal AK, Xing C, DeMartino GN, et al. PSMB8 encoding the beta5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. *Am J Hum Genet.* 2010; 87(6):866–872. [PubMed: 21129723]
61. Brehm A, Liu Y, Sheikh A, et al. Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production. *J Clin Invest.* 2015; 125(11):4196–4211. [PubMed: 26524591]
62. Sanchez GAM, Reinhardt AL, Brogan P, et al. Chronic Atypical Neutrophilic Dermatitis With Lipodystrophy and Elevated Temperatures (CANDLE): Clinical Characterization and Initial Response To Janus Kinase Inhibition With Baricitinib. *Arthritis Rheum-Us.* 2013; 65:S758–S759.
63. Canna SW, de Jesus AA, Gouni S, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat Genet.* 2014; 46(10):1140–1146. [PubMed: 25217959]
64. Romberg N, Al Moussawi K, Nelson-Williams C, et al. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat Genet.* 2014; 46(10):1135–1139. [PubMed: 25217960]
65. Kitamura A, Sasaki Y, Abe T, Kano H, Yasutomo K. An inherited mutation in NLRC4 causes autoinflammation in human and mice. *J Exp Med.* 2014; 211(12):2385–2396. [PubMed: 25385754]
66. Kawasaki Y, Oda H, Ito J, et al. Identification of a High-Frequency Somatic NLRC4 Mutation as a Cause of Autoinflammation by Pluripotent Cell-Based Phenotype Dissection. *Arthritis Rheumatol.* 2017; 69(2):447–459. [PubMed: 27788288]
67. Hu Z, Zhou Q, Zhang C, et al. Structural and biochemical basis for induced self-propagation of NLRC4. *Science.* 2015; 350(6259):399–404. [PubMed: 26449475]
68. Bracaglia C, Prencipe G, De Benedetti F. Macrophage Activation Syndrome: different mechanisms leading to a one clinical syndrome. *Pediatr Rheumatol Online J.* 2017; 15(1):5. [PubMed: 28095869]
69. Canna SW, Girard C, Malle L, et al. Life-threatening NLRC4-associated hyperinflammation successfully treated with IL-18 inhibition. *J Allergy Clin Immunol.* 2016
70. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. The International FMF Consortium. *Cell.* 1997; 90(4):797–807. [PubMed: 9288758]
71. French FMFC. A candidate gene for familial Mediterranean fever. *Nat Genet.* 1997; 17(1):25–31. [PubMed: 9288094]
72. Manukyan G, Aminov R. Update on Pyrin Functions and Mechanisms of Familial Mediterranean Fever. *Front Microbiol.* 2016; 7:456. [PubMed: 27066000]

73. Richards N, Schaner P, Diaz A, et al. Interaction between pyrin and the apoptotic speck protein (ASC) modulates ASC-induced apoptosis. *J Biol Chem*. 2001; 276(42):39320–39329. [PubMed: 11498534]
74. Chae JJ, Cho YH, Lee GS, et al. Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1beta activation and severe autoinflammation in mice. *Immunity*. 2011; 34(5):755–768. [PubMed: 21600797]
75. Xu H, Yang J, Gao W, et al. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature*. 2014; 513(7517):237–241. [PubMed: 24919149]
76. Park YH, Wood G, Kastner DL, Chae JJ. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. *Nat Immunol*. 2016; 17(8):914–921. [PubMed: 27270401]
77. Masters SL, Lagou V, Jeru I, et al. Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. *Sci Transl Med*. 2016; 8(332):332ra345.
78. Kuijk LM, Beekman JM, Koster J, Waterham HR, Frenkel J, Coffey PJ. HMG-CoA reductase inhibition induces IL-1beta release through Rac1/PI3K/PKB-dependent caspase-1 activation. *Blood*. 2008; 112(9):3563–3573. [PubMed: 18684863]
79. Mandey SH, Kuijk LM, Frenkel J, Waterham HR. A role for geranylgeranylation in interleukin-1beta secretion. *Arthritis Rheum*. 2006; 54(11):3690–3695. [PubMed: 17075828]
80. Garcia-Mata R, Boulter E, Burridge K. The 'invisible hand' : regulation of RHO GTPases by RHOGDIs. *Nat Rev Mol Cell Biol*. 2011; 12(8):493–504. [PubMed: 21779026]
81. Goldfinger SE. Colchicine for familial Mediterranean fever. *N Engl J Med*. 1972; 287(25):1302.
82. Mandhare A, Banerjee P. Therapeutic use of colchicine and its derivatives: a patent review. *Expert Opin Ther Pat*. 2016:1–18.
83. Krendel M, Zenke FT, Bokoch GM. Nucleotide exchange factor GEF-H1 mediates cross-talk between microtubules and the actin cytoskeleton. *Nat Cell Biol*. 2002; 4(4):294–301. [PubMed: 11912491]
84. Gao W, Yang J, Liu W, Wang Y, Shao F. Site-specific phosphorylation and microtubule dynamics control Pyrin inflammasome activation. *Proc Natl Acad Sci U S A*. 2016; 113(33):E4857–4866. [PubMed: 27482109]

KEY POINTS

- Two deubiquitinase deficiencies, haploinsufficiency of A20 (HA20) and otulipenia, derive from the impairment of the negative regulation in NF- κ B signaling.
- Deficiency of ADA2 (DADA2) results in clinical manifestations including recurrent lacunar strokes, polyarteritis nodosa-like vasculitis, hypogammaglobulinemia, Diamond-Blackfan anemia, and bone marrow failure.
- STING-associated vasculopathy with onset in infancy (SAVI) is characterized by severe dermatologic and pulmonary lesions.
- Clinical features of NLRC4-related autoinflammatory syndromes vary from cold-induced fever to chronic central nervous system inflammation or macrophage activation syndrome.
- RhoA GTPase suppresses the pyrin inflammasome by stimulating pyrin phosphorylation, which in turn favors the binding of inhibitory 14-3-3 proteins to pyrin. Certain bacterial toxins inactivate RhoA and thereby derepress the pyrin inflammasome. Mutations in MEFV and MVK predispose to autoinflammatory disease by decreasing 14-3-3 interaction with pyrin.

Figure 1. Proposed mechanisms of pathogenesis in deubiquitinase deficiencies

(A) After binding TNF, a signaling complex is recruited to the TNF receptor. The addition of lysine 63 (K63)-linked ubiquitin (Ub) chains to RIPK1 stabilizes its signaling complex and leads to phosphorylation of the IKK complex, degradation of I κ B α , and translocation of the NF- κ B heterodimer into the nucleus. Under normal conditions (left), A20 removes K63-linked Ub chains from the RIPK1 complex and instead conjugates its constituent proteins with lysine 48 (K48)-linked Ub chains to mark them for proteasomal degradation. In haploinsufficiency of A20 (HA20), heterozygous loss of function of A20 results in the

impaired suppressive function of A20, leading to excessive activation of NF κ B signaling (right).

(B) The linear ubiquitin assembly complex (LUBAC) conjugates methionine 1-linked ubiquitin (linear-Ub) to targets including NEMO and RIPK1, which potentiates NF- κ B signaling. OTULIN restricts this signaling by hydrolyzing linear-Ub on LUBAC and its target proteins (left). In otulipenia/OTULIN-related autoinflammatory syndrome (ORAS), biallelic loss of function of OTULIN results in loss of this suppressive function, leading to accentuated activation of NF- κ B signaling (right). For the sake of simplicity, ubiquitination on TRAF2 and TNFR1 are not shown in these figures.

Figure 2. The STING-type I interferon (IFN) pathway

Cytosolic sensing of double stranded DNA (dsDNA) by cyclic GMP-AMP synthase (cGAS) leads to the production of cyclic GMP-AMP (cGAMP) as a second messenger, which then activates the stimulator of interferon genes (STING) in the endoplasmic reticulum (ER) and further upregulates type I interferon (IFN) transcription via the recruitment of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3). The binding of type I IFN molecules to their receptors initiates the transcription of hundreds of IFN-stimulated genes (ISGs) via the activation of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway.

Figure 3. Mechanism of pyrin inflammasome regulation and the diseases caused by pyrin dysregulation

In the resting state Rho family guanosine triphosphate hydrolases (GTPases) are activated by guanine nucleotide exchange factor (GEF). This in turn activates protein kinase N (PKN) 1/2-mediated inactivation of pyrin through its phosphorylation and the recruitment of 14-3-3, a regulatory protein. During certain bacterial infections, RhoA inactivation by bacterial toxins leads to the downstream activation of the pyrin inflammasome by preventing the activation of PKN 1/2, with the resultant dephosphorylation of pyrin and dissociation of 14-3-3. In pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) and familial Mediterranean fever (FMF), mutations of pyrin diminish the binding of 14-3-3 to

pyrin and lead to spontaneous or more easily triggered activation of the pyrin inflammasome.

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Table 1

Newly identified monogenic autoinflammatory disorders.

Disease	Gene	Protein	Phenotypes	Disease Mechanism
HA20	<i>TNFAIP3</i>	A20	Fever, arthralgia, ulcers (oral and genital), and ocular inflammation	Haploinsufficiency of A20 leads to exacerbated NF- κ B signaling and NLRP3 inflammasome activation
Otulipenia/ORAS	<i>OTULIN</i>	OTULIN	Fever, neutrophilic dermatosis, lipodystrophy, arthralgia, diarrhea, and failure to thrive	Loss of OTULIN leads to impaired removal of linear-Ub from proinflammatory signaling complexes
DADA2	<i>CECR1</i>	ADA2	- Fever - Lacunar strokes - Livedo racemosa, ulceration, myalgia - Hepatosplenomegaly, lymphadenopathy - Renal hypertension, aneurysm - Portal hypertension - Anemia, thrombocytopenia, hypogammaglobulinemia	Reduced serum level of ADA2 resulting in: - polarization of macrophages toward M1 (pro-inflammatory) subsets over M2 (anti-inflammatory) - abnormal neutrophil activation - impaired endothelial development - impaired B cell differentiation
SAVI	<i>TMEM173</i>	STING	- Fever - Skin lesions (rash, skin nodules, gangrenous lesions leading to amputation, nail dystrophy/loss, and nasal septum perforation) - Interstitial lung disease with inflammatory infiltrate, fibrosis, emphysematous changes	Gain-of-function in STING leads to the constitutive activation of IFN β signaling
CANDLE	<i>PSMB8</i> <i>PSMB4</i> <i>PSMA3</i> <i>PSMB9</i> <i>POMP</i>	β 5i β 7 α 7 β 1i POMP	Fever, panniculitis, chilblains, lipodystrophy, arthropathy, and brain calcification	Defects in proteasome formation, also associated with upregulation of type I IFN
NLRC4-related autoinflammatory syndromes	<i>NLRC4</i>	NLRC4	Fever, enterocolitis, splenomegaly, and	Gain-of-function in NLRC4 leads to abnormal activation

Disease	Gene	Protein	Phenotypes	Disease Mechanism
			macrophage activation syndrome (NLRC4-MAS) • Cold-induced fever and urticaria (FCAS-like) • Fever, rash, arthralgia, chronic meningitis, brain atrophy, and sensorineural hearing loss (NOMID-like)	of NLRC4 inflammasome, resulting in aberrant production of IL-1β and IL-18, and dysregulation of pyroptotic cell death

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