Lessons from characterization and treatment of the autoinflammatory syndromes

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Purpose of review
The list of genes associated with systemic inflammatory diseases has been steadily growing because of the explosion of new genomic technologies. Significant advances in the past year have deepened our understanding of the molecular mechanisms linked to inflammation and elucidated insights on the efficacy of specific therapies for these and related conditions. We review the molecular pathogenesis of four recently characterized monogenic autoinflammatory diseases: haploinsufficiency of A20, otulipenia, a severe form of pyrin-associated disease, and a monogenic form of systemic juvenile idiopathic arthritis.

Recent findings
The scope of autoinflammation has been broadened to include defects in deubiquitination and cellular redox homeostasis. At the clinical level, we discuss the biological rationale for treatment with cytokine inhibitors and colchicine in respective conditions and the use of interleukin-1 antagonism for diagnostic and therapeutic purposes in the management of undifferentiated autoinflammatory disorders.

Summary
Gene discoveries coupled with studies of molecular function provide knowledge into the biology of inflammatory responses and form the basis for genomically informed therapies. Diseases of dysregulated ubiquitination constitute a novel category of human inflammatory disorders.

Keywords
haploinsufficiency of A20, LACC1, otulipenia, pyrin inflammasome, systemic juvenile idiopathic arthritis

INTRODUCTION

Autoinflammatory diseases are a heterogeneous group of disorders caused by defects in a number of molecules, including inflammasomes, the proteasome, cytokine receptors or inhibitors, and a range of different enzymes [1]. Targeted cytokine therapies are efficacious in most patients, in particular interleukin-1 (IL-1) inhibition [2]. Nonbiological drugs such as the JAK-STAT inhibitors emerged recently. A better understanding of the molecular mechanisms associated with autoinflammation will provide a foundation for developing more affordable and effective treatments for these chronic life-long conditions.

UBIQUTINATINATION-ASSOCIATED AUTOINFLAMMATORY DISEASES

Posttranslational modification by ubiquitination has emerged as a crucial mechanism for regulation of many biological processes, including immune signaling [3]. Ubiquitin (Ub) chains are assembled in response to the activation of innate immune receptors and are attached to target substrates whereby they can modulate protein function. Ub chains exist in different forms, based on linkage type, and they generate distinct cellular signals. Deubiquitinases (DUBs) are enzymes that reverse the effects of ubiquitination by hydrolyzing ubiquitin moieties from modified protein. Genetic alterations affecting DUBs have been associated with immune diseases, human cancers, and neurodegenerative diseases [4]. This past year saw the publication of three articles that linked defects in

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Curr Opin Rheumatol 2017, 29:187–194
DOI:10.1097/BOR.0000000000000362

KEY POINTS

- The discovery of the molecular basis of HA20 and otulipenia suggests a new category of human inflammatory diseases, diseases of dysregulated ubiquitination.
- *MEFV* mutations are associated with a spectrum of autoinflammatory phenotypes including FMF and a severe form of neutrophilic dermatosis (PAAND).
- Pyrin may function as an innate immune 'guard' to detect bacterial virulence induced modifications in the host cell.
- Pyrin mutations might confer heightened responses to infection with *Yersinia* and other pathogens.
- *LACC1*-mediated monogenic Still's disease exemplifies a group of conditions that fall under the label of autoimmuno-inflammatory diseases.

two DUBs to monogenic systemic inflammatory diseases.

HAPLOINSUFFICIENCY OF A20

Zhou *et al.* [5▪▪] described six families with dominantly inherited loss-of-function (LOF) mutations in A20, encoded by the *TNFAIP3* gene, in patients who presented with childhood-onset fevers, athralgia/arthritis, apthous stomatitis, genital ulcers, and ocular inflammation. Clinical manifestations resemble Behcet's disease, which is considered a polygenic/complex disorder. One patient was noted to have features of systemic lupus erythematosus (SLE), including central nervous system vasculitis and idiopathic thrombocytopenic purpura (ITP). Of interest, common variants in the *TNFIAP3* gene have been linked to SLE by genome wide association studies (GWASs) [6,7]. Subsequently, two families of Japanese ancestry have been reported [8,9]. In addition to constitutive symptoms, two patients had severe intestinal inflammation, and were initially diagnosed with entero-Behcet's disease [9].

A20 is an ubiquitin-editing enzyme that plays a key role in the negative regulation of proinflammatory signaling pathways including nuclear factor-κB (NF-κB) [10,11]. This inhibitory function is carried out by two opposite yet synergistic activities, ovarian tumor (OTU) domain-mediated DUB and zinc finger (ZnF) domain-mediated ubiquitin-ligase activity. For example, upon simulation with tumor necrosis factor (TNF), A20 deubiqiuitinates K63 Ub chains on RIPK1 to restrict signaling activity and conjugate K48 Ub chains on RIPK1 to target this protein for proteasomal degradation. All but one disease-associated mutation affects the OTU domain of A20 and they create truncated proteins with defective K63 DUB activity. As a consequence, mutant cells displayed elevated levels of K63 ubiquitinated IKK/NEMO, RIPK1, and TNFR1, which led to activation of downstream signaling complexes (Fig. 1). Patients' primary cells showed constitutive activation of NF-κB and the NLRP3 inflammasome [12,13] and have excessive production of proinflammatory cytokines including IL-1, IL-6, IL-9, IL-17, TNF, IP-10/CXCL10, and IFNγ. Treatment with targeted cytokine therapies, namely IL-1 or TNF inhibitors, attenuates systemic inflammation in these patients.

The disease, haploinsufficiency of A20 (HA20), is so named to reflect the presence of one functional copy of the A20 gene. A complete loss of A20 may not be viable or it could cause a more severe inflammatory phenotype. Low-penetrance common variants, near the *TNFAIP3* gene, have been associated with susceptibility to many autoimmune diseases [14–16]. Given the potent anti-inflammatory function of A20 it has been hypothesized that these susceptibility alleles act as hypomorphic variants. Somatic deletions and biallelic mutations in A20 are found in B-cell lymphomas, which suggested that A20 might also act as a tumor suppressor gene [17]. Germ-line truncating mutations in *TNFAIP3* have not been reported except in patients with HA20.

Mice lacking A20 (*Tnfaip3−/−* mice) [18] exhibit multiorgan inflammation and perinatal death, whereas lineage-specific ablations of A20 give rise to a spectrum of phenotypes resembling human autoimmune conditions [19]. The inflammatory phenotype is particularly severe in A20-deficient dendritic cells. Together, human and murine model studies demonstrate variable and cell-specific effects of rare and common gene variants in A20.

OTULIPENIA/OTULIN-RELATED AUTOINFLAMMATORY SYNDROME

The second disease related to a dysregulation in deubiquitination is caused by recessively inherited LOF mutations in the linear (Met1) DUB OTULIN (also known as *gumby*). OTULIN is a highly conserved cysteine protease with function to specifically hydrolyze linear Ub chains that are assembled by the linear ubiquitin chain assembly complex (LUBAC) [20–22]. Met1 Ub chains play an important role in the propagation of signals in NF-κB, NOD2, and Mitogen-Activated Protein-kinases mediated inflammatory pathways [23]. The LUBAC complex consists of the catalytic subunit HOIL-1-interacting protein (HOIP) (*RNF31*) and two accessory proteins: HOIL-1 (*RBCK1*) and SHARPIN

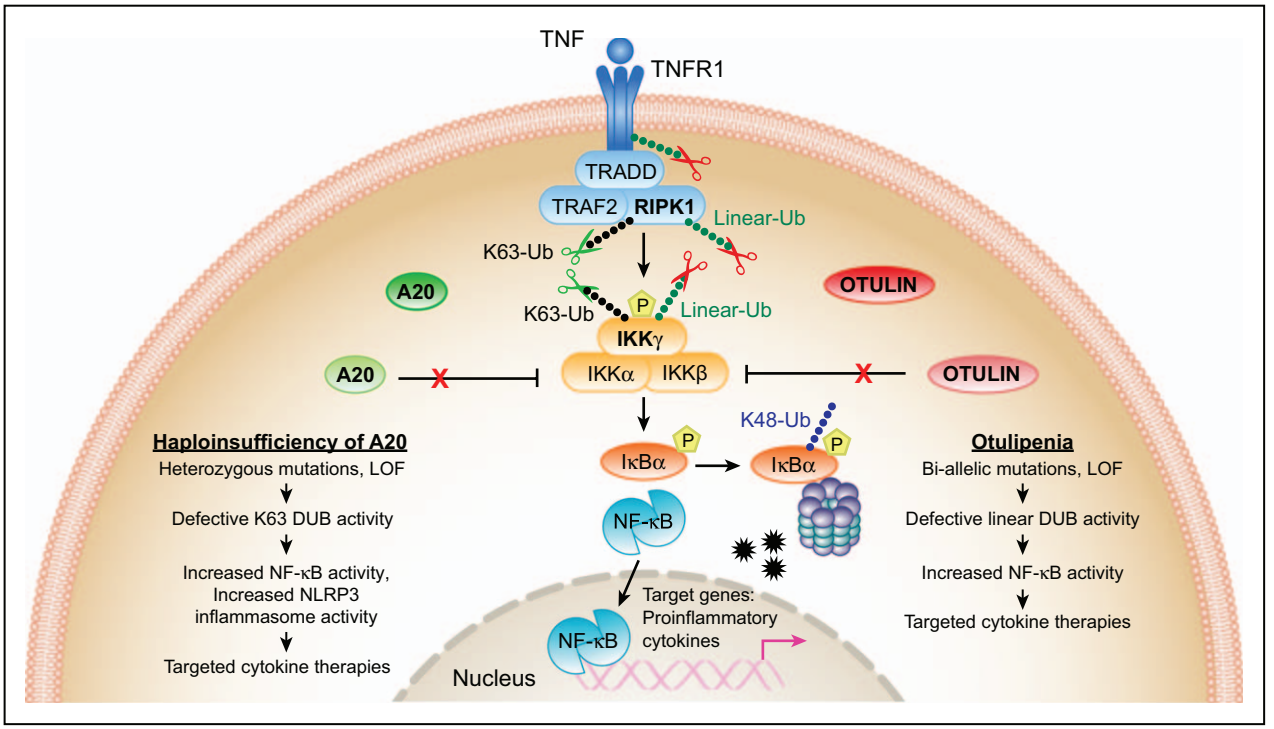

FIGURE 1. Proposed mechanisms of pathogenesis in haploinsufficiency of A20 (HA20) and otulipenia. The canonical NF-κB pathway is regulated both by K63 (Lys63)-linked and linear (Met1)-linked ubiquitin chains. RIPK1 is the central adaptor for assembly of the TNFR1 receptor-signaling complex and is a predominant target for ubiquitination by K63 and linear ubiquitin chains. Polyubiquitylated RIPK1 mediates recruitment of IKK complex that is also target for ubiquitination. The activated IKK complex phosphorylates inhibitor of κB (IκB) and targets IκB for ubiquitin–proteasome system (UPS)-mediated degradation. A20 and OTULIN negatively regulate NF-κB signaling, by cleaving K63 and linear UB chains from target molecules, RIPK1, and IKK. Decreased expression of mutant A20 or OTULIN proteins will lead to activation of the NF-κB pathway and increased expression of proinflammatory transcripts in immune cells. The NLRP3 inflammasome is also negatively regulated by A20 [12,13]. TNFR1, TNF receptor 1; TRADD, TNFR1-associated death domain protein; RIPK1, the death domain-containing protein kinase receptor-interacting protein 1; NLRP3, NACHT, LRR, and PYD domains-containing protein 3.

(*SIPL1*). OTULIN binds to the PNGase/UBA or UBX-containing proteins domain of HOIP and loss of HOIP–OTULIN interaction reduces OTULIN's capacity to restrict LUBAC-induced immune responses [24]. LUBAC-deficient patients have susceptibility to bacterial infections along with episodes of pathogen-free systemic inflammation [25,26]. Mutant cells exhibited impaired NF-κB signaling in fibroblasts and B-cells and enhanced responses to IL-1 stimulation in monocytes, which likely accounts for the features of immunodeficiency and inflammation in these patients.

Zhou *et al.* [27▪▪] reported three unrelated patients of Pakistani and Turkish ancestry with novel homoallelic mutations in the *FAM105B* gene that encodes OTULIN. The disease was termed otulipenia to denote a decreased expression of mutant proteins. Independently, Damgaard *et al.* [28▪▪] described the same Pakistani family and named the disease OTULIN-related autoinflammatory syndrome (ORAS). Patients presented with neonatal-onset severe inflammatory disease manifesting with prolonged fevers, joint swelling, diarrhea, and failure to thrive. Cutaneous features include erythematous or pustular rash, painful skin nodules, and lipodystrophy. Two patients were initially diagnosed with chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE). A skin lesion biopsy showed evidence of neutrophilic dermatitis and panniculitis similar to CANDLE. In contrast to patients with LUBAC-deficiencies, patients with otulipenia have no obvious immunodeficiency. Disease-associated mutations reside in the OTU domain and are predicted to affect binding of OTULIN to linear Ub chains. Consequently, mutant OTULIN proteins failed to prevent the accumulation of linear Ub chains on target substrates IKK/NEMO, RIPK1, TNFR1, and ASC. Similar to HA20, mutant primary cells showed evidence for increased signaling in the canonical NF-κB pathway and overproduction of TNF, IL-1β, IL-6, IL-12, IL-17, IL-18, and IFNγ in serum samples and in response to LPS stimulation (Fig. 1). Anti-TNF therapy is very effective in

normalizing markers of active inflammation, CRP, and ESR, and in controlling disease activity.

Otulin-deficient mice (*gumby*/*gumby*) are embryonic lethal because of compromised angiogenesis and defects in neuronal development [29]. Damgaard *et al.* [28▪▪] showed that cell-specific ablation of Otulin produced viable mice with variable degrees of inflammation. Loss of Otulin in myeloid cells resulted in the most severe phenotype, suggesting a critical role in regulation of the innate immune responses.

In light of these studies, it is interesting to speculate that the DUBs could be legitimate therapeutic targets in the development of novel anti-inflammatory drugs.

PYRIN-ASSOCIATED AUTOINFLAMMATORY DISEASES

The pathogenesis of familial Mediterranean fever (FMF) has been a topic of great interest since the discovery of causal gene *MEFV*, encoding pyrin protein, almost 20 years ago [30]. FMF generally, but not exclusively, affects people of Mediterranean descent and common clinical manifestations include recurrent short-lasting episodes of fever accompanied by painful inflammation of serosal tissues. The symptoms and severity of disease may vary, but typical FMF symptoms correlate with the inheritance of biallelic missense mutations in exon 10 of *MEFV*, which encodes the B32.0 domain. The carrier frequency of disease-associated mutations is above 1% in affected Middle Eastern populations, which raises the question whether *MEFV* variants might be an evolutionary constraint to confer protection against endemic pathogens. Over time, the prevailing paradigm that recessive diseases are caused by LOF mutations has been questioned with reports of numerous patients carrying only one demonstrable disease allele [31–33]. To further complicate matters, mutations outside of exon 10 have been reported in patients with dominantly inherited diseases unlike FMF. The term dominant-FMF was introduced to describe sporadic and familial cases who carry a single mutation most commonly affecting exon 10 residue p.Met694 and may present with late-onset and milder form of FMF [34]. This observation sparked a hypothesis that FMF-associated mutations act as gain-of-function, with a gene–dosage effect, despite the apparent recessive inheritance [35]. This concept has been strengthened by studies in pyrin knock-in mice harboring FMF human mutations [36].

In 2016, Masters *et al.* [37▪▪] reported a distinct clinical phenotype termed pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), which has been linked to a specific mutation, p.Ser242Arg (p.S242R), in exon 2. PAAND is characterized by recurrent long-lasting episodes of fever, prominent skin inflammation including acne, sterile skin abscesses, pyoderma gangrenosum, and arthralgia/mylagia. Cutaneous manifestations closely resemble features seen in patients with PAPA, caused by mutations in the pyrin interacting protein PSTPIP1/CD2BP1. The p.S242R mutation leads to spontaneous activation of the pyrin inflammasome by decreasing binding of pyrin to inhibitory 14-3-3 proteins (Fig. 2, middle). The elucidation of the molecular mechanism in PAAND makes a strong argument that the severity of inflammation in pyrin-associated conditions may be because of the functional impact of *MEFV* mutations on pyrin inhibition.

BEYOND THE PYRIN INFLAMMASOME

In a recent study on the pyrin inflammasome activation Park *et al.* [38▪▪] showed that pyrin inhibition correlates with the activity of RhoA/PKN/14-3-3 signaling cascade. RhoA activation induces PKN-mediated pyrin phosphorylation and binding to 14-3-3 proteins, that in turn inhibits the pyrin inflammasome (Fig. 2, top). Under physiological conditions, bacterial toxins act upon GTPases, such as RhoA, by down-regulating their activity, thereby blocking host responses such as leukocyte migration, phagocytosis, and degranulation that are dependent on RhoA-induced cytoskeletal organization. Thus, it has been proposed that the pyrin inflammasome evolved as an innate immune sensor to detect bacterial-induced modifications of RhoA [39]. This mechanism of sensing bacterial virulence, instead of a direct interaction with pathogen effector proteins, is known as the 'guard mechanism'. Chung *et al.* [40▪▪] recently added an intriguing twist to what appears to be a 'cat and mouse' game between bacterial pathogens and pyrin activation. Their study showed that *Yersinia pestis* effector proteins YopE and YopT activate the pyrin inflammasome by RhoA inactivation, whereas the YopM effector protein negatively regulates pyrin by phosphorylation during infection in macrophages. In light of these data, it is reasonable to hypothesize that FMF-associated gain-of-function mutations might confer a biological and survival advantage for resistance to infection with pathogenic *Yersinia* and other bacteria. Although having a heightened inflammatory response is a high-benefit trait for host defense but possession of two such mutations may become detrimental by triggering an overexuberant immune response.

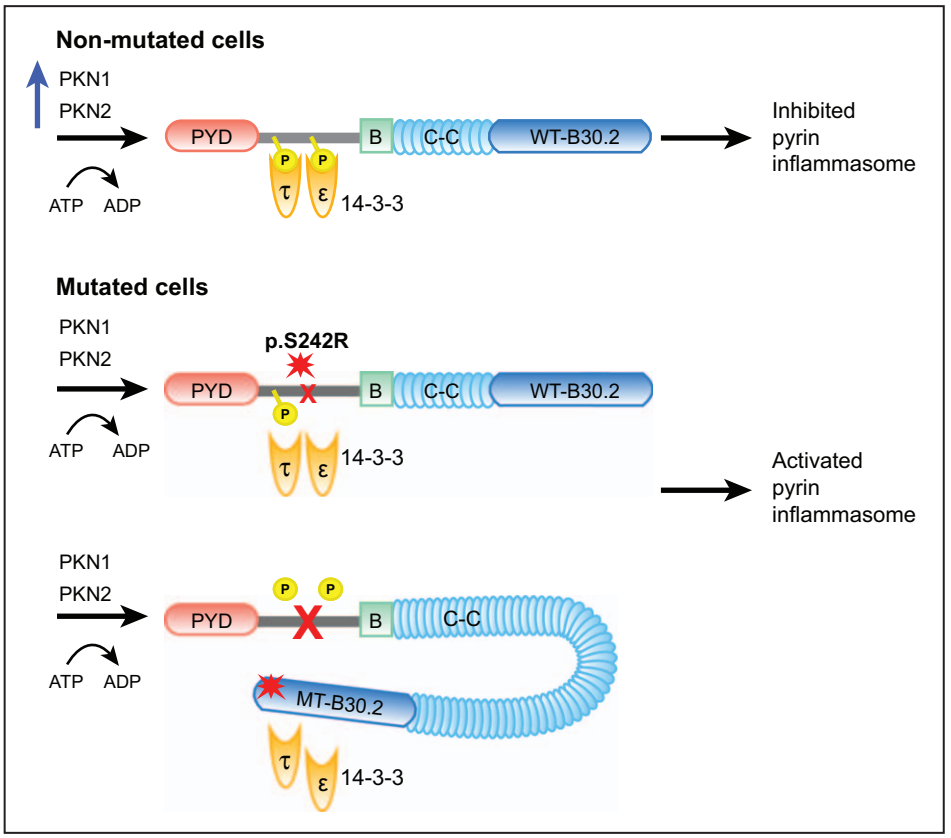

FIGURE 2. Proposed mechanisms of pyrin inflammasome inhibition and pyrin activation in PAAND (pyrin-associated autoinflammation with neutrophilic dermatosis) and FMF (familial Mediterranean fever) diseases. Top panel: the pyrin inflammasome is suppressed following phosphorylation by RhoA effector kinases PKN1/PKN2 and binding to 14-3-3. Middle panel: PAAND-associated p.S242R mutation disrupts the pyrin phosphorylation site, which results in decreased phosphorylation of pyrin, decreased binding to 14-3-3 proteins, and subsequent activation of the pyrin inflammasome. Bottom panel: FMF-associated mutations likely inhibit interaction of pyrin with PKN1/PKN2 and thus cause decreased phosphorylation of pyrin. It is unclear whether the B30.2 domain of pyrin can interact with the protein domain encoded by exon 2. A loop is drawn for the purpose of this figure. Figure adopted from [38▪▪]. PKN1, protein kinase N1; PKN2, protein kinase N2.

Elucidation of the mechanism of pyrin inflammasome regulation also provides new insights into the specificity and effectiveness of colchicine in FMF. Although, there is some uncertainty regarding the precise details, there is an emerging consensus that colchicine is a specific inhibitor of the pyrin inflammasome [38▪▪,41▪▪]. Taken together, these very recent studies on the regulation of pyrin inflammasome activity have answered many questions related to genotype–phenotype correlations, pathophysiology, and efficacy of treatment in patients with pyrin-associated conditions.

LACC1-MEDIATED MONOGENIC STILL'S DISEASE

Systemic juvenile idiopathic arthritis (sJIA) is a highly inflammatory subtype of juvenile idiopathic arthritis (JIA) that typically presents with a characteristic daily (quotidian) spiking fever, and extra-articular features, including erythematous maculopapular rash, symmetric polyarthritis of both small and large joints, which may be variously associated with other disease manifestations, such as serositis, tenosynovitis, and lymphadenopathy.

sJIA had been classified as an autoinflammatory condition until recently, when a meta-analysis of HLA associations in populations of Western European ancestry found that HLA-DRB1*11, and a specific amino acid residue, glutamate 58, was strongly associated with susceptibility to sJIA [$P = 2.7 \times 10^{-16}$, and an odds ratio (OR) of 2.3 (1.9, 2.8)] [42▪]. It has been proposed that sJIA may exemplify a new mode of disease pathogenesis, whereby an essentially autoinflammatory process gradually progresses to an autoimmune phenotype [43]; the term autoimmuno-inflammatory disease has been proposed for such progression [44]. This hypothesis has been further supported by a report of

13 patients with sJIA from five consanguineous families of Saudi Arabian descent carrying a homoallelic missense mutation (p.C284R) in exon 4 of *LACC1* gene, which encodes the enzyme laccase domain-containing 1 [45▪▪]. The cysteine 248 residue is evolutionarily conserved and located in the copper reductase domain of LACC1. This gene has also been associated with leprosy and Crohn's disease, and is now strongly linked to sJIA. Together, these findings implicate *LACC1* in the pathology of autoinflammatory diseases and support the characterization of a subset of sJIA as a recessive monogenic syndrome; however, it should be emphasized that familial sJIA is quite rare and these families represent the exception rather than the rule.

LACC1 is a multicopper oxidoreductase that catalyzes the oxidation of a variety of aromatic substrates, such as phenolic and nonphenolic compounds, with the concomitant reduction of molecular oxygen to water [46]. A study to identify the biological mechanism(s) affected by the C284R and I254V coding variations in LACC1 [47▪▪] found that the protein, renamed as 'FAMIN' ('fatty acid metabolism–immunity nexus') functions as a 'rheostat' for the synthesis of endogenous fatty acids (FAs) and their mitochondrial oxidation and thereby controlled glycolytic activity and overall ATP regeneration. As a consequence, this gene determines the mitochondrial and nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase-dependent production of reactive oxygen species (ROS), bactericidal activity, and NLRP3 inflammasome activation in macrophages. Therefore, sJIA in the Saudi Arabian families described above, has arisen because of a defect at the peroxisome–mitochondrial interface.

Deficiency in mevalonate kinase (MVK), a peroxisomal enzyme in cholesterol metabolism, causes hyper IgD syndrome (HIDS), an autoinflammatory disorder characterized by periodic episodes or 'attacks' of fever associated with joint pain (arthralgia), skin rashes, and abdominal pain. FAMIN was found to complex with fatty acid synthase (FASN) on peroxisomes and promote flux through de-novo lipogenesis (DNL) to drive high levels of fatty-acid oxidation (FAO) and glycolysis, with ATP production [47▪▪]. Interestingly, cytosolic acetyl-CoA, is a shared precursor in common with both DNL and the mevalonate pathway, which suggests the possibility of similar pathogenesis between sJIA and HIDS; furthermore both of these conditions are triggered by vaccinations. A diversity in environmental triggers has been offered to explain how LOF in the same gene may results in two very different disease phenotypes, sJIA, and early-onset Crohn's disease. *LACC1*-mediated monogenic Still's disease is refractory to a range of immunosuppressive agents, including systemic corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), methotrexate, and biologic agents such as adalimumab, etanercept, rituximab, and tocilizumab [45▪▪]. In light of the functional properties of FAMIN, it may be necessary to use small molecule regulation of NLRP3 inflammasome activation, with associated mitochondrial and NADPH-oxidase-dependent production of ROS, to control *LACC1*-mediated disease.

ANAKINRA AS A DIAGNOSTIC CHALLENGE AND TREATMENT OPTION FOR UNDIFFERENTIATED AUTOINFLAMMATORY DISORDERS

The spectrum of poorly defined autoinflammatory disorders that show responsiveness to IL-1 antagonism is now considerable. A recent retrospective case series of 11 adult patients presenting with unexplained pyrexia, serositis, rashes, arthralgia, and other symptoms commonly found in autoinflammatory disorders but without a specific clinical and genetic diagnosis, were ascribed the term undifferentiated systemic autoinflammatory disorder (uSAID) [48▪]. These patients were unresponsive, or only partially controlled, on disease-modifying antirheumatic drug (DMARD)/steroid treatment. Anakinra was found to successfully control symptoms within 4–6 weeks of starting treatment in nine of these 11 cases, with two patients discontinuing therapy. This study demonstrates that anakinra may be a viable treatment option for patients who are unresponsive to standard steroid/DMARD therapies. Moreover, the study suggests that a response to anakinra implicates underlying IL-1 dysregulation in the disease pathogenesis of responding uSAIDs patients.

CONCLUSION

New genomic technologies, high-throughput genotyping (GWAS), and next-gene sequencing (NGS) have advanced the field of systemic inflammatory diseases by identifying novel, rare, or common disease-associated variants that point to pathways underlying these conditions. Animal model investigations further complement human studies by dissecting signaling pathways amenable to targeted therapies. A comprehensive approach to advance understanding of human diseases is exemplified by studies of the A20 protein. Common genetic variants in *TNFAIP3* have been associated with activation of immune responses and cell-survival signals. Linage-specific ablations in mice mimicked human phenotypes and facilitated research on the

function of A20 in specific cell types. Discovery of high-penetrance gene variants informed on the disease mechanism and treatment options in patients with the most severe inflammatory phenotype, HA20. Together, these studies will shed light on understanding the pathogenesis of more common polygenic inflammatory diseases such as rheumatoid arthritis (RA) and celiac disease.

Acknowledgements

The authors would like to thank Dr Daniel Kastner for his helpful suggestions while drafting the manuscript.

Financial support and sponsorship

This work was partially funded by the Cystic Fibrosis Trust (SRC 009).

Conflicts of interest

There are no conflicts of interest.

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