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Monogenetic autoinflammatory diseases: Cytokinopathies

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Abstract

Rapid advances in genetics are providing unprecedented insight into functions of the innate immune system with identification of the mutations that cause monogenetic autoinflammatory disease. Cytokine antagonism is profoundly effective in a subset of these conditions, particularly those associated with increased interleukin-1 (IL-1) activity, the inflammasomopathies. These include syndromes where the production of IL-1 is increased by mutation of innate immune sensors such as NLRP3, upstream signalling molecules such as PSTPIP1 and receptors or downstream signalling molecules, such as IL-1Ra. Another example of this is interferon (IFN) and the interferonopathies, with mutations in the sensors STING and MDA5, the upstream signalling regulator AP1S3, and a downstream inhibitor of IFN signalling, ISG15. We propose that this can be extended to cytokines such as IL-36, with mutations in IL-36Ra, and IL-10, with mutations in IL-10RA and IL-10RB, however mutations in sensors or upstream signalling molecules are yet to be described in these instances. Additionally, autoinflammatory diseases can be caused by multiple cytokines, for example with the activation of NF-κB/Rel, for which we propose the term Relopathies. This nosology is limited in that some cytokine pathways may be degenerate in their generation or execution, however provides insight into likely autoinflammatory disease candidates and the cytokines with which newly identified mutations may be associated, and therefore targeted.

Introduction

In the decade since Hawkins, Lachmann and McDermott described the remarkable efficacy of recombinant human interleukin-1–receptor antagonist anakinra in the treatment of a patient with Muckle-Wells Syndrome[1], the prognosis of patients with cryopyrin-associated periodic fever syndromes (CAPS) has changed dramatically. Prior to the elucidation of IL-1 signalling in CAPS, non-specific immunosuppressive medications were trialled with a relatively poor response. Once the genetic basis was shown to be mutations in *NLRP3*, the gene encoding cryopyrin[2] ,the dominant role of IL-1 in CAPS was established and the theoretical and subsequent practical benefit of anakinra confirmed. Since this time, there has been a focus on determining the genetic basis of inflammatory diseases in general, and exploring potential benefit of biologic agents. Here, we categorise and use monogenetic autoinflammatory diseases to illuminate cytokine pathways, and highlight the complexity and areas of uncertainty in the pathophysiology of these diseases.

IL-1

The role of the IL-1 family in innate and adaptive immunity has been well explored. A total of 11 members have been identified, whose various effects are mediated via four signal receptor complexes and two decoy receptors[3, 4].

The first of these cytokines, IL-1, has many and widespread biological functions including mediation of inflammatory and acute phase responses. The inactive precursor to IL-1β (pro-IL-1β) is found predominantly in the cytoplasm of haematopoietic cells and is produced and activated in response to toll like receptor signalling, complement cascade, cytokines and IL-1 itself [3-7]. Although there is evidence of extracellular cleavage of pro-IL-1β by neutrophil proteinase-3 and elastase, routes of recent interest are both the canonical and non-canonical inflammasome complexes [4, 6].

Sensing

The inflammasome complex formed by NLRP3 (Nalp3, cryopyrin), adaptor protein ASC and caspase-1 senses danger caused by signals such as ATP, amyloid, monosodium urate crystals, calcium pyrophosphate dehydrate crystals and cholesterol crystals. These danger signals lead to opening of ion channels and potassium efflux from cells, with a possible role of changes in intracellular calcium and ROS levels [3-7]. Once activated, the NLRP3 inflammasome cleaves pro-caspase-1 to its active proteolytic form caspase-1 and subsequently cleaves pro-IL-1β to IL-1β[3, 4, 6].

The importance of the inflammasome in IL-1β formation is highlighted in CAPS, a group of diseases with a spectrum of clinical severity. These monogenetic disorders are caused by mutations in *NLRP3*

(also known as *CIAS1*), in regions predominantly coding the nucleotide binding domain [2, 8]. The mutant NLRP3 of these patients exhibits enhanced pro-IL-1β processing activity [8, 9]. Recombinant human interleukin-1–receptor antagonist (Anakinra) is profoundly beneficial for these patients, with reduction in the long term complications of chronic inflammation such as amyloidosis[10]. A human monoclonal antibody targeting IL-1β (Canakinumab) and a dimeric fusion protein that neutralised IL-1β (Rilonacept) are also extremely effective and now have FDA approval for use in the management of patients with CAPS[10, 11].

Despite being one of the earliest autoinflammatory syndromes to be described, the exact pathophysiology of Familial Mediterranean Fever (FMF) is uncertain. The largely autosomal dominant disease is caused by a mutation in *MEFV*, which encodes the pyrin protein[12, 13]. The role of pyrin remains debated and the possibility of both pro-inflammatory and anti-inflammatory effects complicates the understanding of this protein in health and disease. There are a number of theories, including that wild-type pyrin acts as an inhibitor of IL-1β production, or that it is itself prevented from activating IL-1β by various interactions[8, 14, 15]. It has been shown that wild-type pyrin can bind to ASC and make it unavailable for use in the inflammasome, but that it can also form caspase activating inflammasomes[5, 8, 14-16]. It may be the balance of these two functions that is important. There are certainly alternative roles for pyrin as colchicine, a microtubule polymerisation inhibitor, is very effective in the management of FMF. Anti-IL-1 agents, whilst helpful, are usually considered as therapy if response to first line treatment is not complete[8, 10]. Recent data indicates that pyrin is a sensor of bacterial effectors that target RhoGTPases or a factor downstream of this[17]. This suggests that FMF mutations may have been positively selected due to a protective effect against certain species of bacteria that encode these effectors, and that targeting IL-1 may be beneficial during infections where pyrin is activated.

Three recently published papers on clinical syndromes resulting from mutations in *NLRC4* highlight the importance of, and differences between, inflammasome platforms. A mutation resulting in p. Thr337Ser substitution affecting the nucleotide-binding domain of NLRC4 was found on whole exome sequencing of a patient with recurrent febrile and macrophage activating syndrome NLRC4-MAS[18]. This defect leads to constitutive caspase-1 cleavage, and increased secretion of IL-18 from monocytes and macrophages[18]. In the same edition of *Nature Genetics*, a gain in function mutation in *NLRC4* encoding p. Val341Ala in the HD1 domain of the protein leading to a phenotype of enterocolitis and autoinflammation was described[19]. Macrophages from patients with the syndrome of enterocolitis and autoinflammation associated with mutation in *NLRC4* (SCAN4) showed spontaneous formation of ASC foci and increased pyroptosis[19]. In subsequent literature, a

Japanese family with a phenotype consistent with Familial Cold Autoinflammatory Syndrome (FCAS) was shown to have a mutation in *NLRC4* encoding a p. His443Pro substitution in the nucleotide binding domain[20]. Although one patient with NLRC4-MAS has been successfully treated by IL-1 blockade[18], a significant role for IL-18 in this and indeed in the other intrinsic inflammasomopathies cannot be discounted. The variability in the phenotypes of patients with mutations *NLRC4*, and the predominant enteric pathology in the first two descriptions, may yet be explained by IL-18, an effect of commensals, or the NLRC4 bacterial trigger flagellin.

There are a number of regulatory proteins termed NLR family, Apoptosis Inhibitory Proteins (NAIPs) that dictate the specificity of NLRC4 response[21]. NAIP2 is involved upstream of NLRC4 in the recognition of bacterial PrgJ and NAIP5 and 6 respond specifically to bacterial flagellin[21]. In a series of experiments involving transfected cells and combinations of wild-type and constitutively active NAIP5 and NLRC4, it was determined that constitutively active NAIP5 could signal wild-type NLRC4 and hence activate caspase 1[21]. Though there have not been any reports of mutations resulting in gain of function of NAIPs, it is not unreasonable to predict a similar phenotype to those with NLRC4 activating mutations.

The formation of inflammasomes is tightly regulated by proteins with inhibitory or assisting roles. The deubiquitylation of NLRP3 required as an activating step is mediated by BRCC3, however there is no evidence to support gain of function of BRCC3 leading to constitutively activated NLRP3[22]. There are numerous negative regulators of NLRP3 that could be inactivated in autoinflammatory disease[23], for example, nitric oxide (NO), micro-RNAs (miR-223), E3 ligases (TRIM30, March7). The possible involvement of these negative regulators is highlighted by a paper identifying a novel negative regulator of NLRP3 inflammasome activity A20[24]. Myeloid specific deletion of A20 in mice causes increased caspase 1 activation and a phenotype reminiscent of rheumatoid arthritis[24].

Upstream Signalling

There are a number of other autoinflammatory conditions linked to defects upstream of IL-1β, albeit less well defined. Pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome also may involve pyrin and IL-1β, but the association is not exclusive. This autosomal dominant condition results from a mutation in *PSTPIP1* (also known as *CD2BP1*) which encodes proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1)[5, 8, 25]. The mutated PSTPIP1 has stronger and longer binding interaction with pyrin[5, 8, 25]. Depending on whether pyrin is considered pro or anti-inflammatory, there are different explanations of how the mutation causes effect, either through

conformational change to pyrin by the process of binding allowing oligomerisation of pyrin with adapter proteins and formation of an active inflammasome[8]. Alternatively, the mutated PSTPIP1 may prevent the inhibitory function of pyrin on the NLRP3 inflammasome[5]. Whilst most patients experience benefit with IL-1 inhibition, the ongoing flare in some despite high doses suggests more than one cytokine could be involved[5, 8].

Caspase 12, caspase recruitment domain family, member 8 (CARD8), CARD16, CARD17 and CARD18 focus on inhibition of caspase 1 activity[26]. Interestingly, caspase 12 exists in the majority of the population in truncated form, and full length protein due to a single nucleotide polymorphism seen in approximately 20% of African, Asian and South American population renders them hyporesponsive to endotoxin and susceptible to severe sepsis[27]. CARD8 provides negative regulation of NLRP3[28]. CARDs 16, 17 and 18 are induced by pro-inflammatory signals and inhibit caspase 1 activity as part of a negative feedback loop[29, 30]. Furthermore, three pyrin-only proteins (POP1, POP2 and POP3) have been identified and have been shown to inhibit inflammasome formation[31, 32]. Although deficiencies in these CARDs and POPs have not been described in humans, an inflammasomopathy would be a foreseeable consequence.

Receptor or Downstream Signalling

To ensure appropriate homeostasis, there are endogenous antagonists to IL-1 which are secreted in response to pro-inflammatory stimuli. IL-1R antagonist (IL-1Ra) is a naturally occurring competitive inhibitor of IL-1β binding to its receptor[3, 6, 7].

The consequence of lack of inhibition is exemplified in patients with Deficiency of the IL-1 Receptor Antagonist (DIRA), a syndrome characterised by neutrophilic pustular dermatosis, periostitis, aseptic multifocal osteomyelitis, and high acute-phase reactants[33]. These patients have a mutation in *IL1RN*, the gene encoding IL-1Ra[5, 33, 34]. The lack of antagonism leads to unopposed IL-1 receptor activation and increased response to IL-1α and IL-1β. Not surprisingly, these patients show an impressive response to anakinra[10, 33].

Once released, IL-1β binds to a receptor subunit of IL-1R, prompting recruitment of a second receptor subunit. The two TIR domains allow recruitment of myeloid differentiation primary response protein 88 (MYD88), IL-1R-associated kinase 4 (IRAK4), TNFR-associated factor 6 (TRAF6) to initiate nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways[3]. All of these are prime candidates for the identification of activating mutations that drive

autoinflammatory disease, however so far, only loss of function mutations associated with immune deficiency are known.

Unknown

Although the exact pathophysiology is unknown, the shared clinical characteristics between Schnitzler syndrome and CAPS prompted a trial of IL-1β antagonists with great effect[35]. There has been one report of *NLRP3* V198M mutation resulting in the phenotype consistent with the syndrome, but this has not been a uniform finding[36]. Schnitzler syndrome is characterised by a monoclonal IgM gammopathy and features suggestive of an autoinflammatory condition such as fever, arthralgia, lymphadenopathy, hepatosplenomegaly and increased inflammatory markers[8, 35]. Although a number of cytokine disturbances have been reported, clinical response within hours of treatment with anakinra implies that IL-1β is a key player[35]. There are varying reports of the efficacy of TNF-α inhibition in Schnitzler syndrome. A case report of exacerbation of symptoms with adalimumab and etanercept treatment suggested the deterioration in clinical picture after initial improvement was causal[37]. In cases where remission was achieved, longer interval between initiation and resolution of symptoms, as well as the necessity of co-administration with systemic immunosuppressive therapies[38], implies that IL-1β is more directly linked to the pathophysiology and phenotype than TNF-α. More recently, anti-IL-6 treatment with tocilizumab was trialled successfully in three patients with Schnitzler syndrome who failed to respond to IL-1 inhibition[39]. There have been attempts to clarify the pathophysiology of this condition, with studies showing a dominant role for IL-1β and IL-18[40, 41], with anomalous processing and spontaneous release of IL-1β from PBMCs[41]. Whether this is causal, and how this links in with a monoclonal gammopathy remains uncertain.

Majeed syndrome is an autosomal recessive disease caused by a mutation in *LPIN2*, coding protein lipin-2[42, 43]. This protein is a phosphatidate phosphatase (PAP) that is important in glycerolipid biosynthesis, acting as a transcription co-activator regulating lipid metabolism[42, 44] and is upregulated in macrophages during stress[44]. Patients with Majeed syndrome have chronic recurrent multifocal osteomyelitis, inflammatory neutrophilic dermatoses and congenital dyserythropoitic anaemia[45]. Depletion of lipin-2 in murine and human macrophages lead to an increased expression of proinflammatory genes by saturated fatty acids through alteration in phosphorylation of the JNK/c-Jun pathway[44]. However, the full extent of the role of this protein in vivo and the mechanism of the phenotypic consequences of it being defective is not known. Two

patients showed improvement with a trial of IL-1 antagonists but did not respond to anti-TNF-α, linking this disease to the IL-1 pathway by uncertain means[10, 46].

The inflammasomopathies are the paradigm for cytokinopathies, with genetic lesions observed throughout all levels of IL-1β production and signalling, and frequently negative regulators thereof (Figure 1). This can also now be established for the interferonopathies, as described later in this review, however future work may also flesh out a similar network of diseases involving IL-10, or as discussed next, IL-36.

IL-36

The IL-36 cytokines, comprising of IL-36α, IL-36β and IL-36γ, are part of the IL-1 family, agonists exhibiting proinflammatory effects via the IL-36R (IL-1Rrp2)[4, 47]. Once released, IL-36 cytokines lead to a number of factors that induce Th1 and Th17 polarisation[4, 47]. Whilst initially thought to be primarily produced by innate immune cells and lymphocytes, there is evidence of its release from epithelial cells in the skin and lungs as well as brain tissue[47]. Interestingly, despite IL-36R being widely expressed in the brain, studies thus far have failed to show a proinflammatory response with the addition of IL-36β and IL-36γ[48].

Sensing

The expression of IL-36 is tissue dependent. The regulation in skin is controlled by epidermal growth factors and expression in bronchial epithelium enhanced by IL-1, TNF, IL-17 and TLR3 ligands. One potential danger signal triggering IL-36γ production is the alarmin cathelicidin-related antimicrobial peptide (CAMP) LL37[49], however a sensor for this is still controversial.

IL-36 cytokines require processing of the N-terminal methionine to have optimal affinity for IL-36R but the proteases responsible have not yet been elucidated[47, 50]. Such activators would be excellent candidates for activating mutations in autoinflammatory disease.

Upstream Signalling

Some determinants of LL37 signalling have been established, which may be common signalling mechanisms leading to IL-36 upregulation. These include G-proteins and p38[49].

Receptor or Downstream Signalling

The actions of IL-36 are negatively controlled by IL-36Ra, an IL-36R antagonist[4, 47, 50], which blocks recruitment of the second receptor IL-1RAcP (the common co-receptor to IL-1 and IL-33)[4, 47, 50]. Unlike classic antagonists, IL-36Ra has been shown to induce IL-4 mRNA expression and it needs processing for full antagonistic activity[47, 50]. This antagonist is constitutively expressed in keratinocytes[51].

A recently described new autoinflammatory syndrome is that of interleukin-36-receptor antagonist deficiency (DITRA)[33]. Mutations in *IL36RN* result in decreased IL-36Ra antagonistic effects due to defects in both protein stability and affinity for its receptor[33]. The patients with this condition have diffuse pustular erythematous rash associated with high fever, general malaise, systemic inflammation and occasionally a 'geographic tongue' and nail dystrophy[33]. Although there is no established treatment, one patient trialled on anakinra responded positively[52], and various other treatment regimens including corticosteroid therapy, acitretin, and anti-TNF-α have shown some efficacy[33]. The lack of universal response to IL-1 antagonist therapy, as well as the delay of weeks in clinical improvement, suggests that the actions of IL-36 are non-redundant. Whilst there is a positive feedback loop between IL-1 and IL-36, IL-36 also increases TNF and interleukins 6 and 8, and treatment strategies must address these also[53, 54]. Interestingly, a subset of patients with generalised pustular psoriasis (GPP) without preceding psoriasis vulgaris were also noted to have mutations in IL36RN[55, 56], and overexpression of IL-36α in basal keratinocytes in mice lead to acanthosis and hyperkeratosis, highlighting the importance of regulation of IL-36 in keratinocytes[47].

IL-10

IL-10 is widely produced by many immune cells and displays potent anti-inflammatory effects[57-60]. The function of this cytokine is complex, and depends on the cell type on which it is affecting[58-60].

Sensing

In an excellent review of both production and regulation of IL-10, Saraiva and O'Garra detail the complexity of release of IL-10[61]. Both TLR and non-TLR signalling pathways may lead to IL-10 production. They note that the strength of stimuli and the cell on which it acts are variables leading to a different level of IL-10 being released[61].

Upstream Signalling

The predominant signalling pathways leading to IL-10 production are the extracellular signal regulated kinase (ERK), p38 and NF-κB pathways[61]. As these are coincident with both IL-10 and pro-inflammatory cytokines there are no specific autoinflammatory diseases yet known to be caused by mutations in these genes.

There are also a number of pathways leading to the down regulation of IL-10 production. Both the TLR-induced and non-TLR signalling pathways are inhibited by IFN-γ. IL-10 has negative feedback on itself, with expression of Dual Specificity Phosphatase 1 (DuSP1) resulting in decrease in p38 phosphorylation and hence decreased IL-10 production[61]. Again, mutations in these pathways are not yet known.

Receptor or Downstream Signalling

Once released, IL-10 binds to IL-10R, a JAK/STAT3 class of receptor comprised of two subunits IL-10R1 and IL-10R2 encoded by *IL10RA* and *IL10RB* respectively[57, 60]. Once bound to its receptor, the anti-inflammatory effects of IL-10 is dependent on the induction of heme oxygenase-1[60] and eventually inhibits translocation of NF-κB to the nucleus[58]. The downstream effect is inhibition of TLR induced MyD88 translation, STAT1 phosphorylation and INFα and γ gene transcription[58].

Furthermore, association studies of patients with very early onset inflammatory bowel disease (EOIBD) revealed homozygous mutations in *IL10RA* and *IL10RB[60]*. This was replicated in review of patients with early onset enterocolitis, with mutations in *IL10RA* found in 7 of the 14 children developing symptoms before one year of age[62]. These mutations decreased IL-10 signalling with evidence of decreased STAT3 phosphorylation and decreased expression of suppressor of cytokine signalling 3 (SOCS3)[60]. Numerous pro-inflammatory cytokines including but not limited to TNF-α and interleukins 1α, 1β, 2 and 6 were increased[60]. Case reports of bone marrow transplantation resulting in clinical remission have been published[63], but as yet no targeted cytokine treatment option has been shown to be effective in patients with mutations in *IL10RA/IL10RB*. Given the complexity of action of the cytokines, this is not entirely surprising.

Interferons

Interferons have a variety of effects with well described antiviral, antitumor and immunomodulatory activity. Type I interferons (IFN-α, -β, -ω, -ε, -κ) are produced by most cells, whereas NK, NKT and T

cells are the primary sources of type II interferons (IFN-γ)[64]. Type I and type II interferon receptor components (IFNAR1/2 and IFNG1/2 respectively) are expressed on most nucleated cells, suggesting that both have the potential for widespread activity[64].

Sensing

Both TLR dependent and independent pathways may be engaged to induce IFN production. The TLR independent pathway involves retinoic acid inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA5) acting as cytoplasmic sensors of nucleic acid[64, 65]. Furthermore, STimulator of INterferon Genes (STING) and DNA-dependent activator of IFN-regulatory factors (DAI) induce type I IFN in response to cytosolic DNA, either from external pathogens or self[65, 66].

STING is an evolutionarily conserved endoplasmic reticulum transmembrane protein with the downstream effect of activation of IFN regulatory factor 3 (IFR3) transcription factor, translocating to the nucleus and, with NF-κB, transcribes *IFNA* and *IFNB* genes and produce type I IFN[65, 66]. A recently described monogenetic autoinflammatory disorder termed STING-associated vasculopathy with onset in infancy (SAVI) documented early onset vasculitis localised to cheeks, ears, nose, and digits with the absence of thrombocytopaenia and autoantibodies typically associated with antiphospholipid syndrome[67]. These patients were noted to have a gain of function mutation in *TMEM173* gene encoding STING which resulted in a constitutively active STING and positive feedback loop of interferon production and interferon receptor binding[67].

Patients with Aicardi-Goutières syndrome (AGS) have an upregulated IFN gene signature. AGS is a genetically heterogeneous disorder characterised by inflammation of skin and brain, with phenotypes resembling an overlap of sequelae of congenital infection and SLE[68]. Mutations in genes encoding three prime repair exonuclease 1 (*TREX1*), Ribonuclease H2 subunits A, B and C (*RNASEH2A/B/C*), Sam domain- and HD domain containing protein (*SAMHD1*) [68] as well as adenosine deaminase (*ADAR1*) have all been implicated in AGS[69]. Extensive inflammatory features including lymphocytic vasculitis, mouth ulcers, deforming arthropathy and cerebral vasculopathy are seen in patients with SAMHD1 mutations[68]. Both TREX1 and RNASEH2 are nucleases and it is hypothesised that dysfunction of the enzyme activity leads to accumulation of endogenous nucleic acids that are sensed through RIG-I and MDA5 lead to a type I IFN response[68, 70] . Although the exact function of SAMHD1 has not been elucidated, a similar mechanism has been proposed[68].

The role of MDA5 in the immune response and IFN release was highlighted in a mouse model published by Funabiki et al in 2014[71]. Mice with a missense mutation in *IFIH1* leading to G821S in

MDA5 had increase in unstimulated MDA5 signalling leading to upregulation of IFN signature as well as a lupus phenotype[71]. Two case series, one of eleven patients[72] and another of six[73], were published soon after, describing heterozygous mutations in *IFIH1* associated with dysregulation of signalling, increase in IFN and AGS phenotype[72, 73]. Although mutations have not yet been described, similar consequences resulting from gain of function of RIG-1 and LGP-2 may be expected.

Upstream Signalling

Although the exact pathophysiology is still under investigation, patients with spondyloenchondrodysplasia (SPENCD) have elevated type I IFN levels[68, 74, 75]. This disorder is characterised by skeletal dysplasia, cerebral calcification and spasticity, and an increased susceptibility to the development of SLE[68, 74, 75]. SPENCD is caused by biallelic mutations in *ACP5*, a gene encoding tartrate-resistant acid phosphatase (TRAP) [68, 74, 75]. In health, TRAP functions to hydrolyse substrates including nucleotides, phosphoproteins, and osteopontin (Opn). Opn has been shown to have a role in type I IFN production acting as an intracellular signal transduction molecule, and it is hypothesised that patients with defective TRAP have accumulation of Opn and dysregulation of IFN production[68, 74, 75]. TRAP may also have a role in removing nucleic acid that would otherwise have signalled IFN production via RIG-I[68, 74, 75], and thus could be classified with the other sensor mediated inflammasomopathies, however ongoing studies should inform as to the dominant pathway of IFN regulation.

Both SPENCD and AGS patients have a propensity for SLE. Monogenetic forms of SLE have been described with mutations in *C1q, C1r, C1s, C4, DNase1, TREX1 and ACP5* leading to a spectrum of lupus phenotypes[68]. Several lines of evidence exist for the role of IFN in SLE, including the induction of lupus like features in patients on IFN therapy and almost all children with lupus having an upregulated IFN gene signature[68]. However, given the plethora of genes linked to SLE in mouse models and the diverse spectrum of phenotypes, it is likely that the pathogenesis of the disease is complex[68].

Interestingly, a form of monogenetic GPP has been described that disrupts the TLR dependent pathway of IFN induction. A mutation in *AP1S3*, encoding adaptor protein complex 1 (AP1), was noted during a whole exome sequencing of patients with GPP[76]. The destabilised 3D structure of the protein disrupts endosomal translocation of TLR3 and leads to inhibition in downstream signalling[76]. In knockdown keratinocytes, there was a decrease in TLR3 mediated IFN-β induction and subsequent reduction in the anti-inflammatory effects of this cytokine, including failure to

downregulate IL-1[76]. It is the deregulation of IL-1 that is targetable with current medications, with some patients responding to IL-1 blockade[56, 77, 78].

Receptor and Downstream Signalling

Type I IFN acts via heterodimeric type I IFN receptor (IFNAR) which is expressed in most nucleated cells[64]. The dimerization of IFNAR1 and IFNAR2 leads to phosphorylation of TYK2 and JAK1 with subsequent activation of STAT members and transcription of IFN stimulated genes[64]. The downstream result is increased killing of virally infected cells, and increased susceptibility to cell death inducing stimuli[64]. The self-amplification of IFN allows for rapid effect, with type I IFNs increasing expression of molecules that increase IFN production[64]. The potential role of JAK inhibition in limiting this positive feedback loop and hence in treatment of patients with the autoinflammatory disorder SAVI has been explored in vitro, with reduction in *IFNB1* transcription in patients' lymphocytes noted[67]. Further analyses of mouse models may result in clinical trials.

The negative regulation of IFN involves three main processes. Firstly there is downregulation of IFNAR expressed on cell surfaces, achieved through internalisation that is promoted by proinflammatory cytokines, TLRs, as well as oxidative and metabolic stress[79]. Secondly, there is induction of negative regulators SOCS1, SOCS3 and USP18[79]. These mediators are induced by type I IFN as part of a negative feedback loop[79]. SOCS1/3 compete with STAT, while USP18 displaces JAK1, both acting on the IFN receptor[79]. Thirdly, microRNAs are induced and provide regulation of gene transcription[79].

There has been a recent description of three siblings with idiopathic basal ganglia calcification (IBGC), a feature also seen in AGS and SPENCD, with whole exome sequencing determining an autosomal recessive mutation in *ISG15* gene encoding the intracellular ubiquitin-like modifier ISG15[80]. These patients were deficient in ISG15, leading to increased S-phase kinase-associated protein 2 (SKP2) mediated proteolysis of USP18 and subsequently an upregulated IFN gene signature[80]. Prior to this, three unrelated patients with loss of function mutations in ISG15 were reported with a phenotype of disseminated BCG post vaccination attributed with insufficient IFN-γ production[81]. When reviewed, they also demonstrated IBGC and upregulation of IFN-α/β[80].

Multiple

Not all monogenetic autoinflammatory syndromes have their mechanisms linked to one cytokine alone. Despite this, pleiotropic cytokinopathies tend to have a single well defined defect in physiology and can respond remarkably well to targeted therapy.

Sensing

Toll like receptors (TLRs), type I transmembrane receptors with an extracellular domain comprised of leucine rich repeat (LRR) motifs, and a cytoplasmic Toll/IL-1R (TIR) domain, are essential in the eventual transcription of inflammatory related genes through their ability to recognise pathogen associated molecular patterns (PAMPs)[82, 83]. Cell surface TLRs (TLR1, 2, 4, 5 and 6) recognise PAMPs such as LPS, triacyl lipoprotein, diacyl lipopeptides and flagellin[82, 83]. TLRs 2, 4 and 6 have the additional functional capability of recognising danger associated molecular patterns (DAMPs)[83, 84]. A number of endogenous ligands have been elucidated, including but not limited to molecules released from dying cells (HMGB1, heat-shock proteins (Hsp) and extracellular matrix components), amyloid-β, oxidative-LDLs and oxidative-phospholipids[83, 84]. TLRs 7 and 9 recognise self DNA and self RNA respectively[83, 84]. The downstream response of all TLRs except TLR3 is activation of the MyD88 dependent pathway and activation and translocation of NF-κB to the nucleus and alteration in transcription of genes encoding IL-6, IL-12 and TNF[82, 83]. TLR3 along with TLR4 initiate the TIR domain containing adaptor protein inducing IFNB (TRIF) pathway and subsequent type I interferon transcription[82, 83]. Perhaps somewhat surprisingly, monogenic diseases due to upregulation of these elements has not yet been described.

A vital feature in the homeostasis of cells is the recognition of DAMPS released during host stress and injury. An increase in DAMPS, or inability to clear them, will ultimately lead to increase in inflammatory cytokines. The proteasome associated autoinflammatory syndromes (PRAAS) are a group of disorders including chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome, Nakajo–Nishimura syndrome (NNS), joint contractures, muscle atrophy, microcytic anaemia and panniculitis-induced lipodystrophy (JMP) syndrome, Japanese autoinflammatory syndrome with lipodystrophy (JASL), and autoinflammation, LipoDystrophy, and Dermatosis syndrome (ALDD) characterised by severe lipodystrophy and muscle weakness and linked by mutations in *PSMB8*[33, 85, 86]. *PSMB8* encodes the b5i subunit of the immunoproteasome, and mutations in this gene lead to a failure of formation of a complete immunoproteasome. There is subsequent accumulation of DAMPS and hence activation of NF-κB[85, 86], and patients with these disorders have increase in IL-6[85, 86]. There is also constitutively hyper-responsiveness of STAT-1 to IFN and increased IFN signalling, which goes some way to explains why IL-6 antagonists alone have not been effective. JAK inhibitors, targeting the STAT-1

hyperphosphorylation in these patients, and treatment antagonists to the inhibitors of alternative proteolytic proteins are currently being investigated[85].

Despite the name, the clinical features of TNF Receptor-Associated Periodic Syndrome (TRAPS) are not mediated by TNF alone. Patients with TRAPS harbour an autosomal dominant mutation in *TNFRSF1A* which results in abnormally folded TNFR1 [8, 86]. Mutant TNFR1 are retained in the endoplasmic reticulum (ER) and do not progress to function at the cell surface[86]. The receptors may signal in ER during stress and activate JNKs, adding to the transcription of inflammatory mediators[86]. Elevated serum TNF levels are not seen in most patients and there have been reports of infliximab and adalimumab causing inflammatory attacks in this population[10]. There have been positive results with anakinra, with decrease in disease activity and relapse rates, suggesting a role of IL-1 in the pathophysiology of the phenotype. One patient treated with tocilizumab, and IL-6 antagonist, responded[10].

Blau syndrome (BS), a disorder characterised by granulomatous uveitis, arthritis, skin rash, camptodactyly, is caused by an autosomal dominant mutations *NOD2*, encoding the NACHT domain of NOD2 (also known as caspase recruitment domain family member 15, CARD15) and leading to subsequent upregulation of NF-κB activation[8, 87]. It is possible that the mutation leads to a constitutively active NOD2, with further enhanced signalling in response to PAMPs[8, 88]. No single agent has proven effective in patients with BS, TNF inhibition leading to partial control in some, and variable reports of response to IL-1 inhibition[10, 87, 88], highlighting the complexity of this condition and the induction of various cytokines by NF-κB.

The regulation of NF-κB has been explored in experiments involving NLRP12. Long considered to have an inhibitory role on NF-κB, there is ongoing debate as to the mechanism of this effect, as well as possible stimulatory functions[89, 90]. HEK293T cells transfected with wild type NLRP12 demonstrated strong inhibition of NF-κB[91]. Knock out mice studies have implicated NLRP12 as an inflammasome component involved in the recognition of Yersinia Pestis and processing of IL-18 and IL-1β after infection[92]. Canonical pathway of NF-κB activation in patients with NLRP12 mutation and cold induced autoinflammatory disease (NLRP12-associated periodic syndrome or NAPS12) demonstrated altered kinetics rather than peak level of activation, reaching a plateau earlier than healthy counterparts[93]. Possible mechanisms and alternative pathways have been investigated. In $NLRP12^{-/-}$ mice, there was increased non-canonical activation of NF-κB and a phenotype of colitis and increased colon cancer[90]. Another study demonstrated no effect on NF-κB activation, but rather increased ASC speck formation and caspase-1 activation[90]. This suggests a role of the inflammasome and may explain why patients present with the same variability and similar

phenotype to CAPS. Whilst this seems a promising possible pathophysiologic mechanism, the inconsistent response and development of tolerance to IL-1 antagonism suggests that there may be a more complicated and likely dual role of NLRP12[10, 33].

Upstream Signalling

The binding of DAMPs to pattern recognition receptors including TLRs and NLRs triggers a signal cascade involving the adaptor molecules MyD88 and TIR domain-containing adaptor-inducing interferon (TRIF). The end result is activation of NF-κB pathway (via MyD88) and interferon (via TRIF).

HyperIgD syndrome (HIDS) is a disease caused by mutations in *MVK* leading to accumulation of mevalonic acid (MA). In excess, MA is toxic, resulting in deficient biosynthesis, perturbation of signals and defective autophagy[8, 94]. Furthermore, the defective MVK pathway leaves patients unable to produce anti-inflammatory isoprenoids[5, 8]. Decreased isoprenoids has been implicated in increased GTPase Rac1 activity and subsequent activation of caspase-1[5, 8]. Supporting the role of Rac1 are in-vitro studies showing that the inhibition of this enzyme in mononuclear cells from patients with HIDS leads to lower IL-1β production[5, 8, 95]. A number of cytokines have been elevated in patients with HIDS, including IL-1α, IL-1β, TNF-α and IL-6[96]. Recent in vitro studies indicate that the hyperresponsiveness of patients' peripheral blood mononuclear cells and resultant increase in cytokinesis is dependent on stimulation of TLR2, TLR4 and NOD2[96]. Although the TNF-α levels are elevated in these patients, their response to IL-1 inhibition with anakinra is greater than to TNF-α antagonists [10]. Furthermore, a trial of IL-6 antagonist tocilizumab in two patients with ongoing symptoms despite anakinra therapy resulted in clinical improvement in both[96].

The familial form of Pityriasis Rubra Pilaris (PRP) is a rare autosomal dominant condition caused by mutations in *CARD14,* encoding caspase recruitment domain family, member 14 (CARD14) [33, 97]. The gene is specifically expressed in keratinocytes and its protein functions as a regulator of NF-κB signalling. The altered structure of the mutated CARD14 coiled-coil domain increases activation of NF-κB[97], leading to a phenotype of follicular hyperkeratosis, palmoplantar keratoderma, and erythema [33, 97].

Cherubism is an autosomal dominant condition caused by heterozygous mutations in SH3BP2, encoding the signalling adaptor protein SH3-domain binding protein 2 (SH3BP2) [8, 34, 98]. This inflammatory bone condition is characterised by bony swelling of upper and lower jaws, typically with regression during puberty[8, 34, 98]. In health, tankyrase, a member of the polyADP-ribose

polymerase (PARP) family, interacts with SH3BP2 with modulation of downstream pathways. Knockout mice models have demonstrated that mutations in SH3BP2 lead to reduced tankyrase binding, reduced ADP-ribosylation of SH3BP2 and decreased degradation and overproduction of TNF-α[98]. The inflammation in cherubism is thought to be MYD88 dependent, after engagement of TLR2 and TLR4 by DAMPS. One hypothesis for the regression seen with age is the reduction in DAMPS after jaw stabilisation[98]. The limitation to the jaw is difficult to explain. Osteoblasts with this mutation show increased responsiveness to M-CSF and RANKL[8], and in heterozygous mouse models, bone marrow derived M-CSF dependent macrophages have been shown to undergo TNF-α-induced osteoclastogenesis independently of RANKL, through spleen tyrosine kinase (SYK) and phospholipase C γ 2 (PLCγ2) phosphorylation[99]. Interestingly, case reports of patients with cherubism treated with TNF alpha inhibitors did not show significant clinical response, suggesting that TNF alone is not responsible for the phenotype[100].

Activation of and signalling via NF-κB is the downstream effect of multiple cytokines and the perpetuation of the inflammatory response. Linear ubiquitination of NEMO and RIP1 with a polyubiquitin chain generated via linear ubiquitin chain assembly complex (LUBAC) is thought to play a key role in the regulation of the canonical pathway of NF-κB [101, 102]. LUBAC is an ubiquitin ligase (E3) composed of heme-oxidized IRP2 ligase-1 (HOIL-1L, also known as RBCK1), HOIL-1L- interacting protein (HOIP), and SHANK-associated RH domain interacting protein (SHARPIN) [101, 102]. Although much remains to be elucidated with regards to the mechanism of action of the individual components, together in LUBAC, they have a non-redundant role in NF-κB pathway and signalling via TNFR1[101, 102]. LUBAC is abundantly expressed in both thymus and spleen and it is suggested that the complex is required for CD40 and B cell function [101, 102].

In order to elucidate the function of HOIL-1L, a number of experiments with HOIL1$^{-/-}$ mouse model have been documented. However the first report of defect in humans was published in 2012, with loss of function mutations in *HOIL1 (RBCK1)* reported in three individuals from two unrelated families[103]. The phenotype of autoinflammation, invasive bacterial infections, muscular amylopectinosis was due to mutations in HOIL1 leading to LUBAC instability[103]. Interestingly, the effect of this defect varied depending on cell type. NF-κB induction in response to IL-1β and TNF was impaired in fibroblasts. Conversely, monocytes of HOIL-1 deficient patients were hyperresponsive to IL-1β[103] . The susceptibility of these patients to invasive pyogenic infections could be attributed to the impaired response to TLR stimulation. Response to stimulation of TLRs 2, 4 and 6 were partially affected, but IFN-β production via TLR3 was eliminated completely. TLR 7 and 8 stimulation didn't

produce TNF[103]. The mechanism of intracellular glycogen inclusions in their muscles (amylopectinosis) remains to be elucidated.

Receptor and Downstream signalling

Perforin and the cytotoxic CD8+ cells have been shown to be vital in the control of inflammation and underlines the intimate relationship between the adaptive and innate immune system. Genetic defects in this pathway have led to familial haemophagocytic lymphohistiocytosis (fHLH), characterised by expansion of T lymphocytes and macrophages and haemophagocytosis, as well as creation of "cytokine storm"[104, 105]. The proposed mechanism is ongoing antigenic stimulation as a result of impaired killing of infected cells, with a central role of IFN-γ producing CD8+ T cells. Elevated levels of IFN-γ, IL-1β, TNF-α, IL-18 and IL-6 have also been consistently reported[104, 105]. Whilst the mainstay of treatment remains corticosteroid therapy, there have been various results of success and failure with treatment of patients with TNF-α inhibition[104]. Trials of IL-1 and IL-6 antagonism in patients with systemic juvenile idiopathic arthritis (SJIA), a disorder complicated by macrophage activating syndrome (MAS), have been successful[104, 106-108]. The role of these agents in reducing the frequency and/or severity of MAS, or indeed in the treatment of primary HLH is less certain. Case reports of successful resolution of SJIA related MAS after treatment with anakinra and conventional immunosuppression have been published[109-111]. However evidence for use of anakinra monotherapy, canakinumab or tocilizumab in the treatment of MAS is less conclusive[107, 112-115]. Phase II trials looking at tocilizumab and anti-IFN- γ therapy in HLH are currently underway, and results may further assist in elucidating of primary cytokine driving the syndrome[104].

Unknown

Two recently described autoinflammatory conditions involve the PLCγ2. PLCγ2-associated antibody deficiency and immune dysregulation (PLAID) is characterised by cold induced urticaria, granulomatous disease, autoimmune disease and hypogammaglobulinaemia[116, 117]. Patients with this condition have an autosomal dominant inframe deletion in *PLCG2* resulting in gain of function of PLCγ2 and increased PLCγ2-dependent signalling after receptor crosslinking[116, 117]. Chronic stimulation and negative feedback of this pathway may have a role in the pathophysiology of hypogammaglobulinaemia[117]. Autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation (APLAID) patients have a decreased threshold for triggering PLCγ2, without constitutive activation, leading to a phenotype of recurrent blistering skin lesions, bronchiolitis and

recurrent sinopulmonary infections, arthralgia, ocular inflammation, enterocolitis, and mild immunodeficiency without autoantibodies[116, 118]. Like PLAID, APLAID is an autosomal dominant condition caused by a missense mutation in *PLCG2*[116, 118]. Treatment with non-steroidal anti-inflammatory drugs and TNF inhibitors have been unsuccessful and there was only a partial response to an IL-1 inhibition[118]. The mutation in APLAID affects an inhibitory domain of the enzyme leading to enhanced activity and subsequently more intracellular Ca^{++} release and activation of ERK in CD19+ B cells[119]. This increase in intracellular calcium may be a trigger of NLRP3 activation, linking this pathway with inflammasome and IL-1 and possibly explaining a partial response to anti-IL-1 therapy[119].

The H syndrome is characterised by hyperpigmentation, hypertrichosis, hepatosplenomegaly, heart abnormalities, hearing loss, hypogonadism, low height and occasionally hyperglycaemia. It has been linked to mutations in *SLC29A3*, a gene encoding the human equilibrative nucleoside transporter 3 (hENT3), important for passive transport of nucleosides[120]. Patients may also present with recurrent fevers, in addition to classic features of H syndrome[121]. The relationship between severity and SLC29A3 expression is unclear, as is the mechanism of hENT3 dysfunction and phenotype[121]. Cytokines IL-1, IL-6 and TNF are normal in knock out mice, and IL-1 treatments in patients with SLC29A3 spectrum disorders have been unsuccessful[121].

The description of a new autoinflammatory condition linked to deficiency adenosine deaminase 2 (DADA2) was published at the same time as mutations in *CECR1* (encoding ADA2) were linked to familial cases of polyarteritis nodosa[122, 123]. In health, ADA2 has both catalytic and growth factor activities, deactivating extracellular adenosine and terminating signalling through adenosine receptors[122, 124, 125]. Deficiencies in ADA1, an intracellular adenosine deaminase, have been known to cause severe combined immunodeficiency. The phenotype of DADA2 is strikingly different, highlighting the unique roles, with patients with autosomal recessive loss of function *CECR1* mutations presenting with intermittent fevers, systemic inflammation, lacunar strokes, hepatosplenomegaly, and hypogammaglobulinaemia of isotype IgM[123]. These patients had impaired endothelial development and a defect in macrophage development, with abnormal anti-inflammatory M2 differentiation [123]. The mechanism of recurrent fevers and systemic inflammation as well as endothelial dysfunction has not been clarified.

Conclusion

The recent description of unusual phenotypes with features of autoinflammation as well as autoimmunity has blurred the distinction between the two classes of syndromes and has highlighted the intimate link between innate and adaptive immune responses. Furthermore, diseases limited to specific organ systems suggest that autoinflammation need not be systemic. The response to targeted treatment is profound in patients where the specific cytokine involved can be identified. For this reason, it is important to focus on the pathophysiology of diseases, and the dominant cytokine leading to phenotype, to determine whether specific pathways can be targeted to provide therapeutic benefit for patients.

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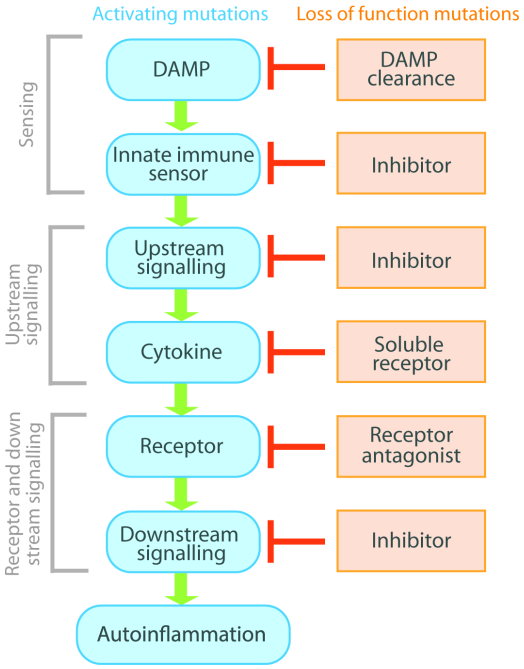
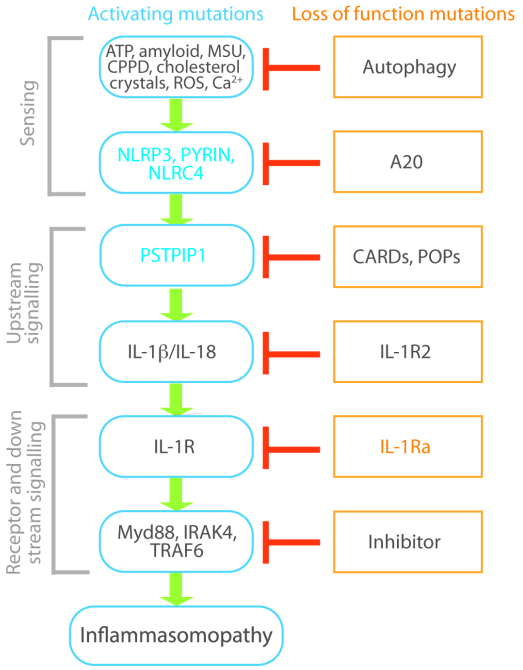
a) Autoinflammatory diseases



b) Inflammasomopathies



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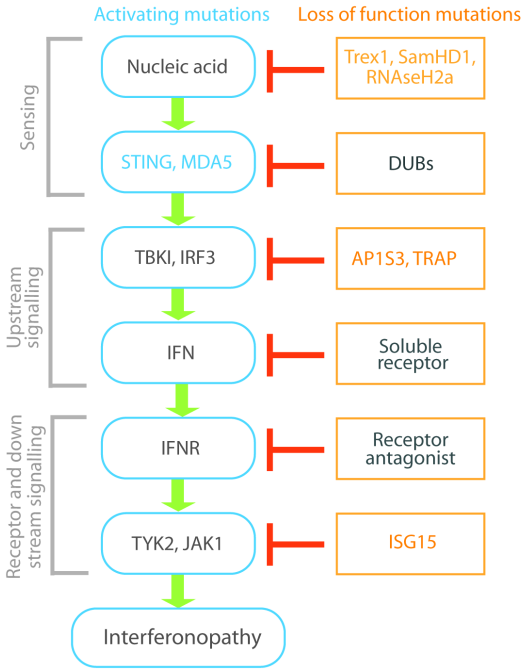

d) Relopathies

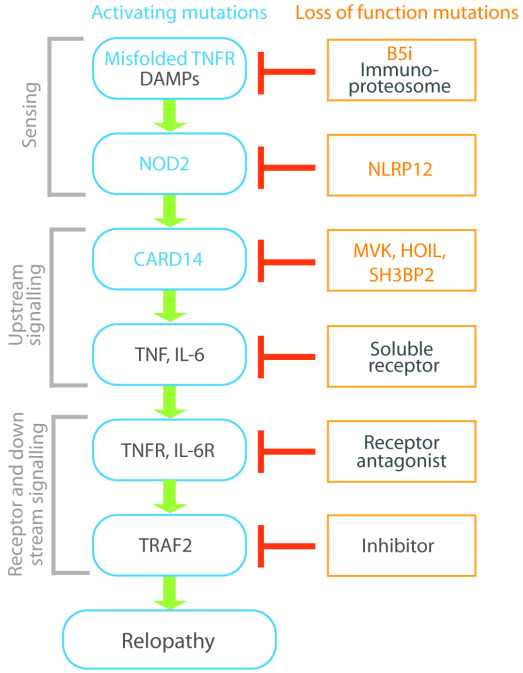

Figure 1. Schematic representation of the Cytokinopathies. (a) A proposed classification to integrate autoinflammatory diseases within a framework of cytokine signalling. (b) This has been applied to the inflammasomopathies, (c) interferonopathies and (d) relopathies, demonstrating examples of proteins that may be involved in autoinflammation. Highlighted in blue are proteins that display activating mutations in human autoinflammatory disease, and in orange those that have loss of function mutations.

DUBs Deubiquitinating enzymes