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Monogenic 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 monogenic 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.

1. 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 monogenic autoinflammatory diseases to illuminate cytokine pathways, and highlight the complexity and areas of uncertainty in the pathophysiology of these diseases (Fig. 1).

2. 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 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 cytoplasmic inflammasome complexes [4,6].

2.1. 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

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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 monogenic 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 recessive 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 regulatory proteins termed NLR family, Apoptosis Inhibitory Proteins (NAIPs) that are involved in the NLRC4 response. Although only one human NAIP has been found, the multiple mouse NAIPs have been shown to dictate specificity for NLRC4 [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].

2.2. 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.