Review TRENDS in Molecular Medicine Vol.13 No.9

From inflammasomes to fevers, crystals and hypertension: how basic research explains inflammatory diseases

Michael F. McDermott[1] and Jürg Tschopp[2]

[1] Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds LS9 7TF, UK
[2] Department of Biochemistry, University of Lausanne, Epalinges, Switzerland

Pattern-recognition receptors, such as Toll-like receptors and NOD-like receptors (NLRs), are able through the recognition of pathogen-associated molecular patterns and danger-associated molecular patterns to sense microbe-dependent and microbe-independent danger and thereby initiate innate immune responses. In some autoinflammatory conditions, abnormalities in NLR signaling pathways are involved in pathogenesis, as exemplified by *NOD2* mutations associated with Crohn's disease. Some other NLRs are components of the inflammasome, a caspase-1- and prointerleukin-1β-activating complex. Clinical and experimental studies are beginning to reveal the central role of the inflammasome in innate immunity. Here, we focus on monogenic hereditary inflammatory diseases, such as Muckle-Wells syndrome, which are associated with mutations in proteins that modulate the activity of the inflammasome, and on some multifactorial disorders, such as Type 2 diabetes and hypertension.

Introduction

It has been appreciated gradually that the structure of germline-encoded pattern-recognition receptors (PRRs) and their recognition of highly conserved pathogen-associated molecular pattern (PAMPs) enables the innate system to discriminate between different types of pathogens and also to generate an appropriate immune response [1]. These PRRs include the Toll-like receptors (TLRs; see Glossary), NOD-like receptors (NLRs), TREM receptors (TREMs), C-type lectin receptors (CLRs) and several other receptors, all of which contribute to immune activation in response to diverse insults, including infection or tissue injury. Collectively, these PRRs are expressed on the cell membrane, in intracellular endosomal compartments or in the cytoplasm.

Compared with all the other innate immune receptor molecules, the NLR pathways appear to be involved predominantly in chronic non-infectious inflammation against self. This has been confirmed in both monogenic and polygenic diseases, in which genetic mutations or functional alterations in NLR-related pathways have been described. The best-known example is Crohn's disease, which is associated with mutations in the NLR member *NOD2* [2]. Interestingly, mutations in the NLR-related system in humans appear to be much more common than all of the other PRRs put together, although the reason for this remains a mystery.

This review will focus on the NALP subfamily of NLRs and will not include a discussion of NODs because numerous excellent reviews on their disease association have been published recently [3,4]. The more recent and rather unexpected associations of NALP members with a plethora of diseases will be discussed.

The NALP3 inflammasome

The NLR system comprises a family of 22 cytoplasmic proteins, which includes five members of the NOD subfamily, 14 NALPs, IPAF, NAIP and CIITA (Table 1). The basic structural features and immunology of the NLR system have been reviewed in detail recently [3,5–9]. The NALPs constitute the largest subfamily of the NLRs, of which the best understood is NALP3 (also called

Glossary

Chronic neurologic cutaneous and articular syndrome (CINCA): also known as neonatal onset multisystem inflammatory disease (NOMID) or NONID; a rare genetic periodic fever syndrome that causes uncontrolled inflammation in multiple parts of the body starting in the newborn period. Symptoms include skin rashes, severe arthritis and chronic meningitis leading to neurologic damage.
FMF: familial Mediterranean fever. A hereditary inflammatory disorder that affects groups of patients originating from around the Mediterranean Sea (hence its name). It is present prominently in the Armenian people (up to 1 in 7 affected), Sephardi Jews (and, to a much lesser extent, Ashkenazi Jews), people from Turkey, the Arab countries and Lebanon.
Hyper IgD syndrome (HIDS): a periodic fever syndrome described originally in 1984 by the internist Professor Jos van der Meer, then at Leiden University Medical Centre. No more than 300 cases have been described worldwide.
MWS: Muckle-Wells syndrome, a rare autosomal dominant disease that causes sensorineural deafness, recurrent hives and can lead to amyloidosis. Individuals with MWS often have episodic fever, chills and painful joints. As a result, MWS is considered a type of periodic fever syndrome.
Toll-like receptors (TLRs): TLRs are single membrane-spanning receptors that recognize structurally conserved molecules derived from microbes. TLRs recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns.

Corresponding author: Tschopp, J. (jurg.tschopp@unil.ch).
Available online 6 September 2007.

 1471-4914/$ – see front matter doi:10.1016/j.molmed.2007.07.005

Table 1. The human NOD-like receptor (NLR) family

NLR subfamily		Domain structure
NALP	NALP1	PYD-NACHT-NAD-LRR-FIIND-CARD
	NALP2	PYD-NACHT-NAD-LRR
	NALP3*	PYD-NACHT-NAD-LRR
	NALP4	PYD-NACHT-NAD-LRR
	NALP5	PYD-NACHT-NAD-LRR
	NALP6	PYD-NACHT-NAD-LRR
	NALP7	PYD-NACHT-NAD-LRR
	NALP8	PYD-NACHT-NAD-LRR
	NALP9	PYD-NACHT-NAD-LRR
	NALP10	PYD-NACHT-NAD
	NALP11	PYD-NACHT-NAD-LRR
	NALP12	PYD-NACHT-NAD-LRR
	NALP13	PYD-NACHT-NAD-LRR
	NALP14	PYD-NACHT-NAD-LRR
NOD	NOD1	CARD-NACHT-NAD-LRR
	NOD2	CARD-CARD-NACHT-NAD-LRR
	NOD3	CARD-NACHT-NAD-LRR
	NOD4	CARD-NACHT-NAD-LRR
	NOD5	X-NACHT-NAD-LRR
IPAF	IPAF	CARD-NACHT-LRR
NAIP	NAIP	BIR-BIR-BIR-NACHT-LRR
CIITA	CIITA	(CARD)-AD-NACHT-NAD-LRR

AD, activation domain; BIR, BIR domain; FIIND, Function to find domain; LRR, Leucine-rich domains; NACHT, NACHT domain; NAD, NACHT-associated domain; PYD, Pyrin domain. NALP3 is frequently called cryopyrin.

cryopyrin), which recognizes numerous exogenous and host ligands [6]. The NALP3 polypeptide consists of three domains: a pyrin domain, a NACHT domain and 11 leucine-rich repeat (LRR) domains (Figure 1). The NALP3 inflammasome, similar to that described originally for NALP1 [10], is a multimeric protein complex that mediates the processing of the proinflammatory cytokine prointerleukin-1β (proIL-1β) [11]. Among its components are NALP3, an adaptor protein called ASC, caspase-1 and Cardinal, which is thought to be a functional homologue of the C-terminus of NALP1. Activated caspase-1 cleaves a 116 amino acid region from the N-terminus of the cytosolic proIL-1β (p35) to convert it to the active form, interleukin (IL)-1β (p17) [12]. The precursor forms of IL-18 and probably IL-33 [13] are similarly dependent on processing by caspase-1. IL-1β is a pyrogenic cytokine that is normally activated in response to infection, injury and immunological challenge [12]; it is produced mainly by blood monocytes and, at minimal concentrations, causes fever, hypotension and the production of additional proinflammatory cytokines, such as IL-6.

As yet, little is known about the activators and modulators of the NALP3 inflammasome activity. The LRRs of NALP3 can detect the bacterial component peptidoglycan (PGN) [14]; however, activation of the NALP3 inflammasome is also induced by endogenous, microbe-independent stress signals. For instance, the exposure of macrophages to ATP or monosodium urate (MSU) crystals induces strong activation of caspase-1 in a NALP3 inflammasome-dependent manner [15,16]. The sensing of these ligands leads to the oligomerization of NALP3 (Figure 1), followed by the recruitment of ASC and caspase-1, leading to formation of an active inflammasome complex. Inflammasome activity is dampened by several cytoplasmic proteins, including pyrin [17,18], which is a protein that is associated with the autoinflammatory disease, familial Mediterranean fever (FMF, see later) [19].

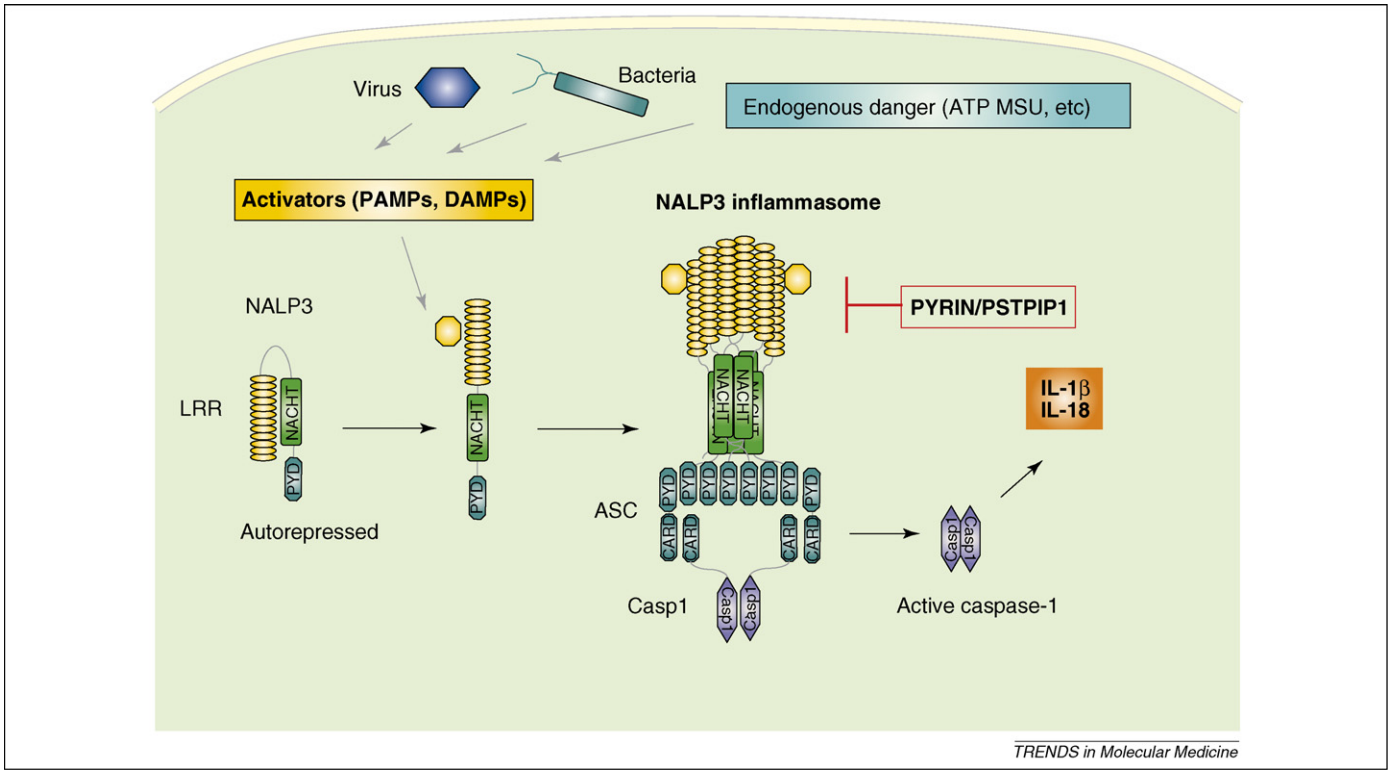

Figure 1. Processing of interleukin (IL)-1β through the NALP3 inflammasome. The initially autorepressed NALP3 is activated through viral (RNA), bacterial (PGN, toxins) (PAMPs) or endogenous components (uric acid crystals, MSU, ATP) (DAMPs). Activated NALP3 oligomerizes and then recruits ASC and caspase-1, which results in caspase-1 processing and activation. Caspase-1 then cleaves and thereby activates the precursor forms of IL-1β and IL-18 (and possibly IL-33). Inflammasome activity is inhibited by pyrin and its interaction partner PSTPIP1. CARD, CARD domain; LRR, Leucine-rich domain; NACHT, NACHT domain; PGN, Peptidoglycan; PYD, Pyrin domain.

Despite all these discoveries, relatively little is known about the production of NALPs. Most of these proteins are secreted in cells of lymphoid or myeloid origin, although significant production of some NALPs is also found in sperm or oocytes. Granulocytes, monocytes, dendritic cells, B and T cells all produce NALP1 and NALP3 [20] and the highest levels of NALP1 are found in T cells and Langerhans cells. Furthermore, NALP1 is present in glandular epithelial structures, such as stomach, gut, lung, and, surprisingly, in neurons and testis. In contrast to NALP1, NALP3 shows a more restricted tissue distribution with secretion mainly in non-keratinizing epithelia in the oropharynx, oesophagus and ectocervix. Moreover, NALP3 production is found in the urothelial layer in the bladder [20].

The hereditary periodic fevers: central role of the NALP3 inflammasome

The blanket term, hereditary periodic fevers, has been applied to a group of distinct heritable disorders, characterized by unexplained episodes of fever and severe localized inflammation [21]. The membranous synovial and serosal linings are particular targets, leading to articular and abdominal pain; in addition, these patients might suffer from rashes and varying degrees of neurological involvement. The hereditary periodic fevers are included in the larger family of systemic autoinflammatory diseases that differ from autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), in that they lack high-titre autoantibodies or antigen-specific T cells.

MWS, FCAS, CINCA

Muckle-Wells syndrome (MWS; see Table 2), familial cold autoinflammatory syndrome (FCAS) and chronic infantile cutaneous neurologic articular syndrome (CINCA; also called neonatal-onset multisystem inflammatory disease, NOMID), which are all caused by mutations in the *CIAS1* gene (also called *NALP3*), encoding NALP3, are hereditary periodic fever syndromes that are considered as a spectrum of disorders with overlapping characteristics. The severity and duration of the attacks vary from a few days to several weeks in these disorders. MWS and FCAS develop in childhood generally; FCAS (described previously as familial cold urticaria [FCU]) is associated typically with the development of fevers, skin lesions, arthralgia and conjunctivitis, following exposure to cold. Although amyloidosis is sometimes observed in FCAS, it is much more commonly associated with MWS, together with sensorineural deafness and rash. CINCA is the most severe of the three conditions, with neonatal onset, recurrent arthralgia and, significantly, chronic polymorphonuclear meningitis, which in turn might lead to a gradual increase in neurological impairment in approximately 50% of cases. As with MWS, progressive deafness might develop with age, although CINCA can be fatal in childhood [22].

The disease-causing mutations in the *CIAS1* gene link MWS, FCAS and CINCA into one family of disorders: the 'pyrinopathies' or cryopyrin-associated periodic syndromes (CAPS). Thus, what was thought initially to be three distinct disorders is in fact a representation of a spectrum of clinical symptoms, with FCAS being the mildest, MWS being of intermediate severity and CINCA being the most

Table 2. Inflammasome and NLR components mutated or activated in autoinflammatory diseases

Autoinflammatory disease	Gene mutated	Excess activator	Signalling pathway affected	Response to anakinra
Muckle-Wells syndrome (MWS)	*NALP3*		Inflammasome	Yes
Familial cold autoinflammatory syndrome (FCAS, FCU)	*NALP3*		Inflammasome	Yes
Chronic infantile neurological cutaneous and articular syndrome (CINCA, NOMID)	*NALP3*		Inflammasome	Yes
Familial Mediterranean Fever (FMF)	*Pyrin*		Inflammasome	Partial
Pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA)	*PSTPIP1*		Inflammasome	Yes [31]
Vitiligo	*NALP1* [38]		Inflammasome?	?
Gout		MSU	Inflammasome [15]	Yes [37]
Pseudogout		CPPD	Inflammasome [15]	?
Contact dermatitis		Irritants Trinitro-chlorobenzene	Inflammasome [46,47]	?
Hyperimmunoglobulin D syndrome	Mevalonate kinase		Inflammasome?*	Yes [48]
Systemic-onset juvenile idiopathic arthritis (SOJIA)	?		Inflammasome?*	Yes [49]
Adult-onset Still's disease	?		Inflammasome?*	Yes [50,51]
Behçet's disease	*IL-1β* polymorphism [52]		Inflammasome?*	Yes
Schnitzler's syndrome	?		Inflammasome?*	Yes [53]
Blau syndrome	*NOD2* [54,55]		RIP2–NF-κB	?
Crohn's disease	*NOD2* [54,55]		RIP2–NF-κB?	No [56]
Other diseases				
Hydatidiform mole	*NALP7* [41]		Inflammasome?	?
Hypertension	*NALP3* [43]		Inflammasome?	?

*The involvement of the inflammasome is suggested by the successful treatment of the disease by the IL-1 receptor antagonist anakinra.

severe. The majority of mutations, clustered within the highly conserved NACHT domain of NALP3 (Figure 2), are thought to result in spontaneous caspase-1 activation with IL-1β production and fever [11]. Aksentijevich *et al.* expanded the panel of CINCA-causing mutations and further supported the preliminary observation that the majority of mutations in these patients might have arisen *de novo* [23]; it is likely that this phenotype is sufficiently severe as to impair reproductive fitness.

Consistent with the role of NALP3 in IL-1β regulation, an increase in IL-1β processing was found in the one CINCA patient studied [23]. Although CINCA-related mutations are also located in the same NACHT region, these are distinct mutations, with the exception of *D303N,* which has also been found in MWS (Figure 2). Most mutations tend to cluster into distinct areas around the NACHT region, including the Mg^{2+}-coordinating aspartate at residue 303, which suggests that they might alter

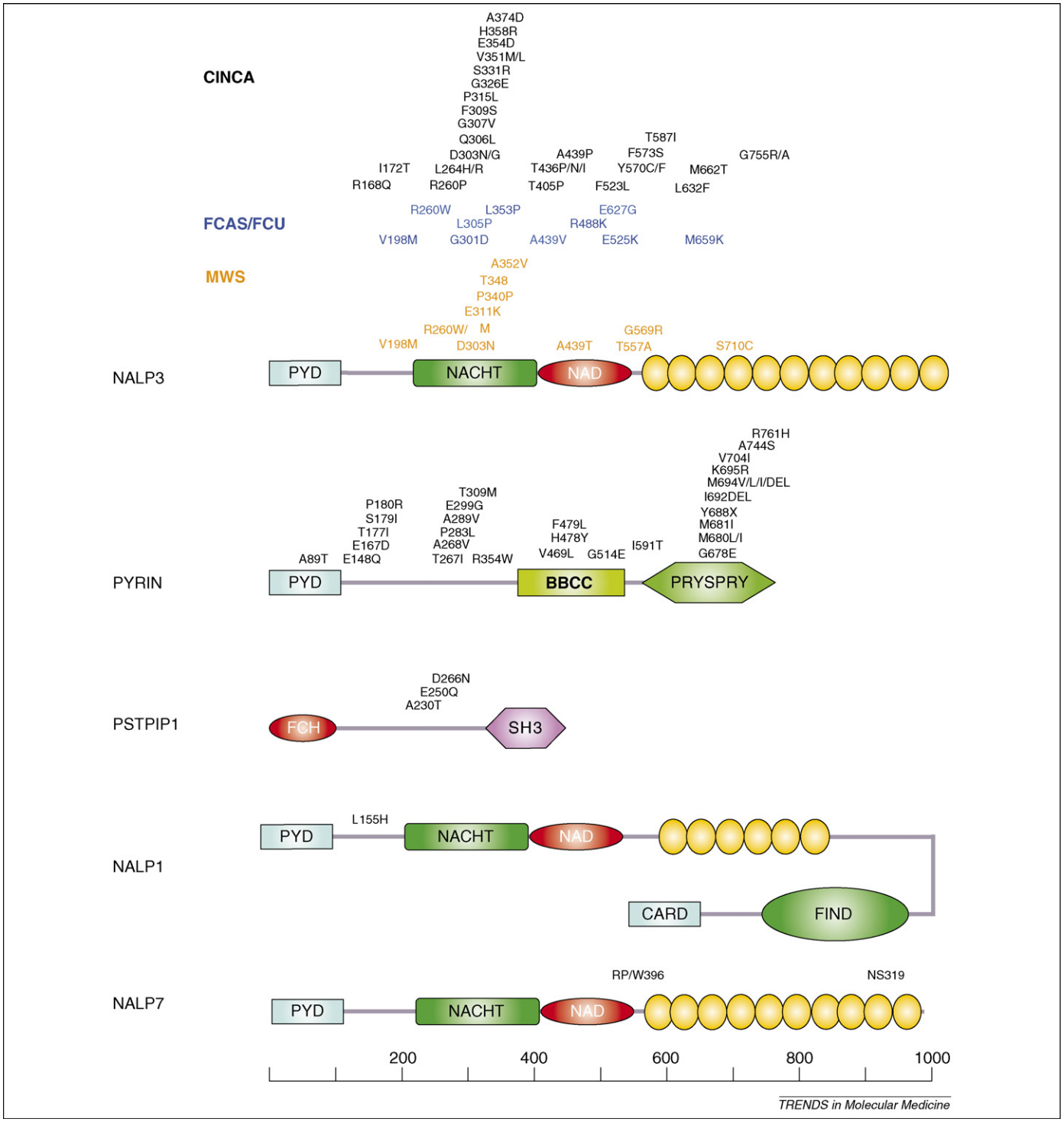

Figure 2. Mutations in proteins associated with inflammasome-related autoinflammatory diseases. Domain architecture and mutations found in disease-associated NALP3 (MWS, FACS, CINCA), Pyrin (FMF), the pyrin-interacting PSTPIP1 (PAPA), NALP1 (vitiligo) and NALP7 (hydatidiform mole). The mutations shown are listed in the Infevers database (http://fmf.igh.cnrs.fr/ISSAID/infevers/).

the function of this domain. The NACHT domain is thought to mediate the oligomerization of NALP3 and, thus, it is likely that those mutations associated with the inflammatory diseases are evoking spontaneous oligomerization (Figure 1). Somatic mosaicism of *CIAS1* has been reported in a patient with CINCA [24] and Fujisawa *et al.* have shown that CINCA-related mutations induce cathepsin B-dependent cell death of human THP-1 monocytic cells [25]. These findings have important implications for laboratories involved in screening for these conditions.

Owing to the genetic basis of these disorders, IL-1β antagonism was proposed as a suitable strategy for therapy because there was no acceptable treatment previously. Indeed, the use of anakinra (IL-1ra, a natural IL-1 antagonist) in all three conditions has led to the most striking and dramatic improvements in symptoms [26–28]. Therapy with anakinra has proved to be life-saving when MWS is complicated by secondary amyloidosis; Hawkins *et al.* reported three MWS patients with serum amyloid A levels over 140 mg l^{-1}, in whom all features associated with active inflammation ceased within just 4 hours of the first subcutaneous injection of anakinra in each subject and plasma serum amyloid A concentrations fell to less than 3 mg l^{-1} in all subjects after 7 days and remained below this level for the following 3 months [26].

Hoffman *et al.* showed that pretreatment of FCAS patients with anakinra blocked clinical and serological symptoms completely following an experimental cold challenge [28]. Anakinra also produced improvement in the most severe form of the pyrinopathies, namely CINCA. Renal secondary amyloidosis, severe arthropathy and neurological complications are long-term disabilities associated with this disease, which has been difficult to treat because steroids do not control the disease completely yet expose the patient to harmful side effects. Several studies have demonstrated a dramatic clinical change post-anakinra therapy, with disappearance of fatigue, rash, conjunctivitis and arthralgias [27,29,30]. Because the life expectancy of CINCA patients is reduced, the long-term effects of anakinra on the mental and physical development of these children will be intriguing. It is hoped that early and prolonged treatment with anakinra might prevent the deafness and abnormal neurological and skeletal development [27]. Anakinra is also an effective therapy for flares of pyogenic sterile arthritis, pyoderma gangrenosum and acne syndrome (PAPA) disease (see later), however, so far, this has only been reported in one case [31].

FMF and PAPA

FMF, inherited as an autosomal recessive trait, is the most prevalent hereditary fever syndrome worldwide and the mutated FMF gene (designated *MEFV* from Mediterranean Fever) is expressed predominantly in granulocytes, activated monocytes and in early leukocyte development [32]. The biological function of pyrin (also called marenostrin; the FMF protein) is the subject of intensive research and a consensus regarding its function is being achieved gradually. Pyrin contains at least four different conserved domains, comprising an N-terminal pyrin domain, a B-box zinc-finger, a coiled coil and a PrySpry domain (Figure 2). Pyrin was found initially to bind to ASC protein, which is the adaptor protein found in the NALP3 inflammasome complex (Figure 1), through the pyrin domain (PYD), suggesting a function for pyrin in the control of inflammasome activity.

Indeed, evidence that pyrin has an anti-inflammatory role comes from targeted disruption of pyrin in the mouse, which shows an increase in IL-1β processing and defective lipopolysaccharide (LPS)- and IL-4-induced apoptosis in peritoneal monocytes [18]. Most human mutations are localized in the PrySpry domain at the C-terminal end of pyrin (Figure 2). Interestingly, this very PrySpry domain interacts with caspase-1, proIL-1β and NALP3, thereby inhibiting the activity of the NALP3 inflammasome [17,33]. Human cells treated with Pyrin siRNA or shRNA exhibited increased release of IL-1β on stimulation of the inflammasome [17,33], which is similar to the effects reported in Pyrin-deficient mice. In one study, a pyrin molecule containing the FMF-associated PrySpry mutations showed reduced caspase-1 interaction and decreased efficiency in blocking IL-1β release [17].

Further information about the function of pyrin has resulted from identification of the gene for PAPA, which is a rare autosomal dominant disorder characterized by recurrent inflammatory episodes, with accumulation of sterile, pyogenic, neutrophil-rich material in the joints. It is caused by mutations of the proline serine threonine phosphatase-interacting protein 1 (PSTPIP1) (Figure 2); this protein is involved in actin reorganization during cytoskeletal-mediated events and interacts with pyrin through its SH3 domain (Figure 2). The effects of PAPA-associated mutations on pyrin binding and downstream pathways are not understood completely, although it is tempting to speculate that the disease-associated mutations might somehow affect the interaction between pyrin and PSTPIP1 and thus modulate inflammasome activity indirectly. The pathophysiology of PAPA is the result of a marked increase in the interaction of PSTPIP1 with pyrin owing to tyrosine hyperphosphorylation, which is regulated by a PEST-type protein tyrosine phosphatase (PTP) [34]. Mutations in *PSTPIP1* prevent the interaction between PSTPIP1 and PTP and thus abolish dephosphorylation of PSTPIP1, resulting in increased interaction with pyrin. Thus far, the only associated mutations to have been reported in *PSTPIP1*, *A230T*, *E250Q* and *D266N*, are located at the PTP–PEST binding site [35].

Another recognized NLR-related disease is Blau syndrome (BS), a rare autosomal dominant disorder characterized by granulomatous polyarthritis, panuveitis and rash [36]. Although the pathogenesis does not appear to involve the NALP3 inflammasome, it is interesting to note that mutations in the NACHT domain of the NLR-member NOD2 protein have been associated with this condition. It has been suggested that BS mutations lead to a gain-of-function mutation in NOD2 proteins resulting in excessive signalling and constitutive NF-κB activation.

Gout and pseudogout

Genetic, molecular and clinical studies of rare hereditary autoinflammatory disorders provide compelling examples of the translation of basic research into advances in clinical

practice, with possible applications to many more common autoinflammatory diseases, such as gout. Gout is an arthritic condition that arises from abnormal purine metabolism associated with deposition of MSU crystals in joints and periarticular tissues. Likewise, pseudogout arises from the deposition of calcium pyrophosphate dihydrate (CPPD) crystals owing to unknown factors. Some of the molecular mechanisms underlying both MSU- and CPPD-induced inflammation have been elucidated by Martinon *et al.* who demonstrated that MSU and CPPD crystals activate the NALP3 inflammasome, resulting in the production of active IL-1β and IL-18 [15]. IL-1β inhibition reduced MSU crystal-induced inflammation in a mouse model [15], because of decreased peritoneal neutrophil accumulation; therefore, a pilot study was performed in ten patients with gout who had failed standard anti-inflammatory therapies. All patients responded to 100 mg of anakinra daily for 3 days [37].

NALP1 and vitiligo

Variants of *NALP1* on chromosome 17 have been implicated in the susceptibility to several autoimmune diseases, including vitiligo [38]. This same genomic region also appears to contribute to SLE susceptibility in members of families who inherit lupus together with vitiligo [39]. There is an increased frequency of several other autoimmune and autoinflammatory diseases, particularly autoimmune thyroid disease (Graves' disease and autoimmune hypothyroidism), Addison's disease, RA, psoriasis, pernicious anaemia and SLE among patients with generalized vitiligo. It is possible that an interplay between both innate and adaptive immune systems are important determinants in the eventual disease expression of vitiligo.

NALP7 and hydatidiform mole

The term hydatidiform mole (HM) describes a human pregnancy without an embryo but with cystic degeneration of chorionic villi [40]. The common form of this condition occurs once in every 1500 pregnancies in Western societies and at a higher incidence in some other geographic regions. Recurrent moles account for approximately 2% of all cases and some of these occur in more than one family member. The non-inherited form of HM usually occurs because of the presence of two paternal genomes in the embryo or placenta, whereas the rare familial cases of recurrent biparental complete HM (BiCHM) moles possess sets of both maternal and paternal chromosomes. A recessive maternal locus was mapped to chromosome 19q13.4 for BiCHM susceptibility and causative mutations identified in the NALP7 protein [41]. The exact role of NALP7 in the pathophysiology of molar pregnancies is unknown but it could have a role in oogenesis or in the endometrium during trophoblast invasion and decidualization.

NALP 14 and spermatogenic failure

Because of the common use of intracytoplasmic sperm injection and the potential genetic aetiology of spermatogenic failure, concern has been raised about transmitting genetic disorders to intracytoplasmic sperm injection offspring. However, so far only in ~15% of all cases of spermatogenic failure can an underlying genetic cause be identified. Previously, an association between spermatogenic failure and chromosomal region 11p15 was established. A mutation screen revealed five mutations in *NALP14* that occurred only in the patient group [57]. One of these unique mutations introduced an early stop codon in the *NALP14* sequence, predicted to result in a severely truncated protein. These data suggest that NALP14 has a function in spermatogenesis and that mutations in this gene might cause spermatogenic failure [57].

Hypertension and Type 2 diabetes

Recently, an exciting link between Type 2 diabetes and inflammasome activity has begun to be unravelled [42]. Pancreatic β cells producing IL-1β have been observed previously in pancreatic sections obtained from patients with Type 2 diabetes and, depending on culture conditions, high glucose levels increased β-cell production and the release of IL-1β, followed by functional impairment and apoptosis. These findings suggested that intra-islet production of inflammatory mediators has a role in the pathogenesis of Type 2 diabetes and that IL-1β was a potential therapeutic target for preserving β-cell mass and function in patients with this condition. Indeed, the blockade of IL-1β with anakinra in patients with Type 2 diabetes improved glycaemia and β-cell secretory function considerably and reduced markers of systemic inflammation.

There is compelling evidence that hyperinsulinaemia and hyperglycaemia in Type 2 diabetes is associated with hypertension. Inflammation and oxidative stress have emerged as major factors in vascular remodelling of hypertension. Polymorphonuclear leukocytes are the main producer of reactive oxygen species (ROS) and are involved in the pathogenesis of hypertension in both human and animal models. Thus, the observation that *NALP3* is mutated frequently in patients with essential hypertension might help to explain why insulin resistance and increased blood pressure often occur together [43].

Predisposition to inflammatory diseases is associated with NLR family members

The interplay between PRRs and, in particular, between TLR and NLR pathways, and the consequent effects on innate and subsequently adaptive immune responses is becoming an area of intensive research. The NLRs are all expressed intracellularly, either in the cytoplasm or the nucleus, unlike the TLRs, which are all found either in plasma membranes and/or endosomal vesicles [20]. Therefore, TLRs recognize extracellular or compartmentalized microorganisms. Many of these extracellular microbes act as commensals, however, and do not necessarily constitute a threat to the host. Only if damage to the cell membrane, cell invasion or metabolic changes in the local microenvironment is detected, are the microbes considered to be dangerous. The NLRs, and in particular the NALPs, are able to detect intracellular microbial products, such as muramyl dipeptide, which signal danger to the host by microbial invasion [14,44]. Furthermore, the detection of

non-microbial molecules, including ROS [45], ATP [16] or crystals [15], endows the NALP system with the ability to sense microenvironmental changes due to oxidative and/or metabolic stress, often caused by invading pathogens. This enables coordinated TLR and NLR activation, whereby stimulation of TLR and NLR pathways leads to enhanced transcription of inflammatory cytokines, such as proIL-1β, through the NALP3 inflammasome. This scenario of sequential activation of TLRs and NLRs suggests that TLRs might detect the early presence of microorganisms and warn of potentially dangerous invaders, however, it is the activation of NLRs following cellular damage that activates the totality of innate immune responses.

Concluding remarks

In recent years, the study of inflammatory disease has progressed from genetics of autoinflammatory conditions to definition of the functional defects in these patients. The majority of hereditary periodic fevers are due to presumed gain-of-function mutations in the pyrin and tumour necrosis factor (TNF) receptor superfamilies of molecules; both pyrin and NALP3 proteins interact with the ASC adaptor protein involved in caspase-1 activation and IL-1β release (e.g. in CINCA). In other conditions, inflammasome activation might arise owing to locally produced, tissue-derived ligands (e.g. ROS in hypertension and uric acid crystals in gout). Although a direct association between defective innate immune responses to bacterial components and conditions, such as Crohn's disease, has not been proven, much work is being done with the goal of confirming that hypothesis.

In any case, the newly discovered involvement of the inflammasome and IL-1β in several autoinflammatory diseases has not only enabled us to further dissect signalling pathways in inflammation but has also provided the conceptual basis for clinical trials aimed at treating autoinflammatory diseases, such as MWS, FCAS and CINCA or gout. As a result, the quality of life of hundreds of patients with these devastating inflammatory diseases has been improved. Several novel inhibitors of IL-1β are currently under development and some have already entered clinical testing in patients with these various diseases, as proof of principle. Considering that the inflammasome was only identified in 2002, the pace by which this discovery in basic research was translated into clinical applications is quite astonishing (Box 1).

Box 1. Outstanding questions

- Why do the *NALP3* mutations, found in inflammatory diseases, render the inflammasome more active?
- Why are so many inflammatory diseases associated with mutations in NLR genes and less with TLR gene mutations?
- How do NALP3 activators activate the inflammasome? Is it a direct or indirect contact?
- How does extracellular uric acid activate cytoplasmic NALP3?
- What is the role of NALP7 mutations in molar pregnancies?
- Do NALP4–12 form a proIL-1β processing inflammasome? What is their role?
- Will IL-1β inhibitors be successful in the treatment of skin diseases or hypertension?

Acknowledgements

This work was supported by the Swiss Committee for Technology and Innovation, MUGEN and the Sir Jules Thorn Charitable Trust (UK).

References

1 Akira, S. *et al.* (2006) Pathogen recognition and innate immunity. *Cell* 124, 783–801
2 Hugot, J.P. (2006) CARD15/NOD2 mutations in Crohn's disease. *Ann. N. Y. Acad. Sci.* 1072, 9–18
3 Fritz, J.H. *et al.* (2006) Nod-like proteins in immunity, inflammation and disease. *Nat. Immunol.* 7, 1250–1257
4 Baumgart, D.C. and Carding, S.R. (2007) Inflammatory bowel disease: cause and immunobiology. *Lancet* 369, 1627–1640
5 Meylan, E. *et al.* (2006) Intracellular pattern recognition receptors in the host response. *Nature* 442, 39–44
6 Martinon, F. and Tschopp, J. (2007) Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* 14, 10–22
7 Ting, J.P. and Davis, B. (2005) CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu. Rev. Immunol.* 23, 387–414
8 Inohara *et al.* (2005) NOD–LRR proteins: role in host–microbial interactions and inflammatory disease. *Annu. Rev. Biochem.* 74, 355–383
9 Mariathasan, S. and Monack, D.M. (2007) Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat. Rev. Immunol.* 7, 31–40
10 Martinon, F. *et al.* (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. *Mol. Cell* 10, 417–426
11 Agostini, L. *et al.* (2004) NALP3 forms an IL-1β processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 20, 319–325
12 Dinarello, C.A. (1998) Interleukin-1 β, interleukin-18, and the interleukin-1 β converting enzyme. *Ann. N. Y. Acad. Sci.* 856, 1–11
13 Schmitz, J. *et al.* (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23, 479–490
14 Martinon, F. *et al.* (2004) Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr. Biol.* 14, 1929–1934
15 Martinon, F. *et al.* (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440, 237–241
16 Mariathasan, S. *et al.* (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232
17 Chae, J.J. *et al.* (2006) The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1β production. *Proc. Natl. Acad. Sci. U. S. A.* 103, 9982–9987
18 Chae, J.J. *et al.* (2003) Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. *Mol. Cell* 11, 591–604
19 Samuels, J. *et al.* (1998) Familial Mediterranean fever at the millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. *Medicine (Baltimore)* 77, 268–297
20 Kummer, J.A. *et al.* (2007) Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. *J. Histochem. Cytochem.* 55, 443–452
21 Kastner, D.L. (2005) Hereditary periodic fever syndromes. *Hematology Am. Soc. Hematol. Educ. Program* 74–81
22 Neven, B. *et al.* (2004) Molecular basis of the spectral expression of *CIAS1* mutations associated with phagocytic cell-mediated autoinflammatory disorders CINCA/NOMID, MWS, and FCU. *Blood* 103, 2809–2815
23 Aksentijevich, I. *et al.* (2002) *De novo CIAS1* mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. *Arthritis Rheum.* 46, 3340–3348
24 Saito, M. *et al.* (2005) Somatic mosaicism of *CIAS1* in a patient with chronic infantile neurologic, cutaneous, articular syndrome. *Arthritis Rheum.* 52, 3579–3585

25 Fujisawa, A. *et al.* (2007) Disease-associated mutations in *CIAS1* induce cathepsin B-dependent rapid cell death of human THP-1 monocytic cells. *Blood* 109, 2903–2911
26 Hawkins, P.N. *et al.* (2003) Interleukin-1-receptor antagonist in the Muckle-Wells syndrome. *N. Engl. J. Med.* 348, 2583–2584
27 Goldbach-Mansky, R. *et al.* (2006) Neonatal-onset multisystem inflammatory disease responsive to interleukin-1β inhibition. *N. Engl. J. Med.* 355, 581–592
28 Hoffman, H.M. and Firestein, G.S. (2006) Anakinra for the treatment of neonatal-onset multisystem inflammatory disease. *Nat. Clin. Pract. Rheumatol.* 2, 646–647
29 Hawkins, P.N. *et al.* (2004) Response to anakinra in a *de novo* case of neonatal-onset multisystem inflammatory disease. *Arthritis Rheum.* 50, 2708–2709
30 Thornton, B.D. *et al.* (2007) Successful treatment of renal amyloidosis due to familial cold autoinflammatory syndrome using an interleukin 1 receptor antagonist. *Am. J. Kidney Dis.* 49, 477–481
31 Dierselhuis, M.P. *et al.* (2005) Anakinra for flares of pyogenic arthritis in PAPA syndrome. *Rheumatology (Oxford)* 44, 406–408
32 Centola, M. *et al.* (2000) The gene for familial Mediterranean fever, *MEFV*, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. *Blood* 95, 3223–3231
33 Papin, S. *et al.* (2007) The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1β processing. *Cell Death Differ.* 14, 1457–1466
34 Shoham, N.G. *et al.* (2003) Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13501–13506
35 Wise, C.A. *et al.* (2002) Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. *Hum. Mol. Genet.* 11, 961–969
36 Pastores, G.M. *et al.* (1990) Autosomal dominant granulomatous arthritis, uveitis, skin rash, and synovial cysts. *J. Pediatr.* 117, 403–408
37 So, A. *et al.* (2007) A pilot study of IL-1 inhibition by anakinra in acute gout. *Arthritis Res. Ther.* 9, R28
38 Jin, Y. *et al.* (2007) NALP1 in vitiligo-associated multiple autoimmune disease. *N. Engl. J. Med.* 356, 1216–1225
39 Nath, S.K. *et al.* (2001) Evidence for a susceptibility gene, *SLEV1*, on chromosome 17p13 in families with vitiligo-related systemic lupus erythematosus. *Am. J. Hum. Genet.* 69, 1401–1406
40 Slim, R. and Mehio, A. (2007) The genetics of hydatidiform moles: new lights on an ancient disease. *Clin. Genet.* 71, 25–34
41 Murdoch, S. *et al.* (2006) Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. *Nat. Genet.* 38, 300–302
42 Larsen, C.M. *et al.* (2007) Interleukin-1-receptor antagonist in Type 2 diabetes mellitus. *N. Engl. J. Med.* 356, 1517–1526
43 Omi, T. *et al.* (2006) An intronic variable number of tandem repeat polymorphisms of the cold-induced autoinflammatory syndrome 1 (CIAS1) gene modifies gene expression and is associated with essential hypertension. *Eur. J. Hum. Genet.* 14, 1295–1305
44 Faustin, B. *et al.* (2007) Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol. Cell* 25, 713–724
45 Petrilli, V. *et al.* Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentrations. *Cell Death Differ.* (in press)
46 Sutterwala, F.S. *et al.* (2006) Critical role for NALP3/CIAS1/cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* 24, 317–327
47 Watanabe, H. *et al.* (2007) Activation of the IL-1β-processing inflammasome is involved in contact hypersensitivity. *J. Invest. Dermatol.* 127, 1956–1963
48 Bodar, E.J. *et al.* (2005) Effect of etanercept and anakinra on inflammatory attacks in the hyper-IgD syndrome: introducing a vaccination provocation model. *Neth. J. Med.* 63, 260–264
49 Pascual, V. *et al.* (2005) Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J. Exp. Med.* 201, 1479–1486
50 Rudinskaya, A. and Trock, D.H. (2003) Successful treatment of a patient with refractory adult-onset still disease with anakinra. *J. Clin. Rheumatol.* 9, 330–332
51 Fitzgerald, A.A. *et al.* (2005) Rapid responses to anakinra in patients with refractory adult-onset Still's disease. *Arthritis Rheum.* 52, 1794–1803
52 Coskun, M. *et al.* (2005) Specific interleukin-1 gene polymorphisms in Turkish patients with Behcet's disease. *Exp. Dermatol.* 14, 124–129
53 de Koning, H.D. *et al.* (2006) Beneficial response to anakinra and thalidomide in Schnitzler's syndrome. *Ann. Rheum. Dis.* 65, 542–544
54 Ogura, Y. *et al.* (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411, 603–606
55 Hugot, J.P. *et al.* (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411, 599–603
56 Carter, J.D. *et al.* (2003) Crohn disease worsened by anakinra administration. *J. Clin. Rheumatol.* 9, 276–277
57 Westerveld, G.H. *et al.* (2006) Mutations in the testis-specific NALP14 gene in men suffering from spermatogenic failure. *Hum. Reprod.* 21, 3178–3184