



## Review Article

## Autoinflammatory diseases - a new entity of inflammation

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The autoinflammatory diseases are characterized by seemingly unprovoked episodes of inflammation, without high-titer autoantibodies or antigen-specific T cells. The concept was proposed ten years ago with the identification of the genes underlying hereditary periodic fever syndromes. NLRP3 inflammasome activation and IL-1 $\beta$  secretion have recently emerged as a central mechanism in the pathogenesis of disease. Here we describe four genetically defined syndromes like cryopyrin-associated periodic syndromes (CAPS, cryopyrinopathies), mevalonate kinase deficiency (MKD) or hyper-IgD and periodic fever syndrome (HIDS), pyogenic aseptic arthritis, pyoderma gangrenosum, and acne syndrome (PAPA syndrome), and deficiency of interleukin-1-receptor antagonist (DIRA) along with the pitfall for understanding the pathophysiology.

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## Introduction

Inflammation has evolved as a physiologic mechanism necessary to defend our bodies from external and internal danger triggers such as infectious agents, chemical factors, and physical factors<sup>1)</sup>.

The innate immune system is assigned to recognize and encounter these stimuli. Recently, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) have emerged as key players for the proper accomplishment of this process through recognition of pathogen associated molecular patterns (PAMPs)<sup>2)</sup>. In addition to PAMPs, NLRs also sense endogenous stress signals known as damage associated molecular patterns (DAMPs)<sup>2,3)</sup>. NLR dependent recognition of either exogenous or endogenous danger signals

initiates the recruitment of adaptor proteins and the formation of molecular platforms referred to as inflammasomes<sup>2, 3)</sup>. In other word, inflammasomes are cellular alerts that assemble in response to microbial invasion and/or cellular damage and alert the system by triggering an inflammatory response. The subsequent activation of caspase-1 results in the post-transcriptional, proteolytic modulation of the related cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18 from their precursor to their active and secreted form, enhancing the inflammatory process. Among several NLRs that form inflammasome platforms, the most studied are NALP1, NALP3 (NLRP3) and IPAF<sup>2, 3, 4)</sup>.

The identification of the critical role of NLRP3 inflammasome in the maturation of these inflammatory cytokines prompted the study of its role in the pathogenesis of several syndromes. The term IL-1 $\beta$  dependent autoinflammatory syndromes has been adopted for such syndromes. This group of diseases is characterized by defective regulation of innate immune response and the absence of autoantibodies or antigen-specific T cells<sup>5)</sup>.

Dysregulation of NLRP3 inflammasome based on mutations of inflammasome related genes has been implicated in the pathogenesis of cryopyrin-associated periodic fever syndrome (CAPS), hyper-IgD syndrome (HIDS), pyogenic arthritis, pyoderma gangrenosum, and acne syndrome (PAPA), or deficiency of IL-1 receptor antagonist (DIRA)<sup>6)</sup>. Interestingly, NLRP3 inflammasome activation by danger signals such as monosodium urate (MSU), calcium pyrophosphate dehydrate (CPPD), amyloid-beta, glucose or silica and asbestos is proposed

as a key molecular mechanism in diseases including gout, pseudogout, Alzheimer's disease, pulmonary fibrosis or the 2 diabetes mellitus<sup>5)</sup>.

We discuss in this review about this new-coming entity of diseases along with the pitfall for understanding the pathophysiology.

## NLRP3 inflammasome

Recognition of microorganisms by the innate immune system depends on conserved germ line-encoded receptors called pattern-recognition receptors (PRRs) that sense conserved motifs present on microbes named PAMPs<sup>7)</sup>.

PRRs are classified into three groups: secreted, trans-membrane and cytosolic (Fig. 1). Secreted PRRs such as collectins, ficolins and pentraxins bind microbes and activate the complement system. The trans-membrane PRRs are Toll-like receptors (TLRs) and the C-type lectins, with some members expressed on cell surface (such as TLR2/4 and Dectin1/2) and some expressed on endosome membrane (TLR3/7/9). The cytosolic PRRs include the RIG-I-like receptors (RLRs), the nucleotide-binding domain leucine-rich repeat containing receptors (NLRs) and the newly identified DNA sensors AIM2 (absent in melanoma 2) and IFI16 (interferon-inducible protein16)<sup>8,9,10)</sup>. Although the RLRs mainly detect viral pathogens, the NLRs can detect both PAMPs and DAMPs<sup>11)</sup>. In response to PAMPs or DAMPs, a subset of NLRs forms a complex with ASC (apoptosis-associated speck-like protein containing a CARD) to activate caspase-1<sup>12)</sup>. In 2002, Tschopp group first named this complex the inflammasome<sup>13)</sup>. Up to date, at least 4 different inflammasomes have been identified; they are the NLRP1, NLRP3, IPAF (NLRC4) and AIM2 inflammasomes<sup>14)</sup>.

NLRP3, also called CIAS1, PYPAF1, Cryopyrin, CLR1.1 (CATERPILLAR 1.1) or NALP3, is one of the best characterized NLR family members. In mice, NLRP3 is mainly expressed in tissues such as lung, liver, kidney, colon and ovary, with particularly high expression in the skin and eye<sup>15, 16)</sup>. Mouse neutrophils, peripheral blood mononuclear cells (PBMCs) and bone marrow-derived dendritic cells (BMDCs) express high level of NLRP3 constitutively, while the bone marrow-derived macrophages (BMDMs) and Th2 cells only express this molecule at moderate level<sup>15, 16)</sup>. However, upon TLR or TNF receptor stimulation, the expression of NLRP3 in BMDMs is dramatically elevated, largely as a result of NF- $\kappa$ B



activation<sup>16, 17</sup>). Both mouse and human osteoblasts express NLRP3<sup>18</sup>). In addition, primary human PBMCs, the monocyte-derived THP-1 cell line, primary human keratinocytes (PK), keratinocyte-derived HaCaT cells, primary mast cells (MS), granu-

locytes and B cells all express NLRP3<sup>19, 20, 21</sup>). The tissue distribution of human NLRP3 is also found in the urothelial layer in the bladder and in epithelial cells lining the oral and genital tracts besides the skin cells mentioned above<sup>20,21</sup>).

secreted

collectin  
ficolin  
pentraxin

trans-membrane

Toll-like receptor (TLR)  
C-type lectin

cytosolic

RIG-I-like receptor (RIR)  
nucleotide-binding domain leucine-rich repeat containing receptor (NLR)  
absent in melanoma 2 (AIM2)  
interferon-inducible protein 16 (IFI16)

### Fig. 1 Classification of pattern-recognition receptors

Recognition of microorganisms by the innate immune system depends on conserved germ line-encoded receptors called pattern-recognition receptors (PRRs) that sense conserved motifs present on microbes named PAMPs.

PRRs are classified into three groups: secreted, trans-membrane and cytosolic.

Structural analysis revealed that NLRP3 contains an N-terminal pyrin domain, an intermediate NACHT domain and a C-terminal LRR domain. Upon activation, NLRP3 recruits ASC via a pyrin-pyrindomain interaction and the recruited ASC binds to pro-caspase-1 via a CARD-CARD interaction. The multi-protein complex thus formed, now called the NLRP3 inflammasome, then activates caspase-1, and the latter cleaves pro-interleukin-1 $\beta$  (IL-1 $\beta$ ) and pro-IL-18 to form mature IL-1 $\beta$  and IL-18, respectively (Fig. 2)<sup>22</sup>). Whole pathogens, PAMPs, DAMPs and environmental irritants can all activate the NLRP3 inflammasome. However, the exact mechanism(s) leading to NLRP3 inflammasome activation is still not clear. Given the diversity of these NLRP3 activators, a consensus is emerging that there is a common downstream intracellular activator that constitutes a final common pathway for NLRP3 activation (Fig. 2)<sup>23,24</sup>). In any case, the mature IL-1 $\beta$  and IL-18 production resulted from NLRP3

activation are highly potent proinflammatory mediators important for host defense against infectious agents. In addition, via IL-1 $\beta$ , the NLRP3 inflammasome is linked to Th17 cell differentiation<sup>25,26</sup>) as well as to Th2 response since vaccination with aluminum adjuvants also activates this inflammasome<sup>27,28</sup>). It should be noted, however, that NLRP3-mediated secretion of IL-1 $\beta$  and IL-18 must be under tight control, as excessive production of these cytokines can lead to autoinflammatory diseases. This is seen in the group of diseases collectively called cryopyrin(CIAS1, NLRP3)-associated periodic syndromes (CAPSs) which are caused by hyper-activation of the NLRP3 inflammasome due to mutations in the NLRP3 gene<sup>29,30</sup>). Besides, hyperimmunoglobulinemia D with periodic fever syndrome (HIDS), the deficiency of the IL-1 receptor antagonist (DIRA), and the syndrome of pyogenic arthritis with pyoderma gangrenosum and acne (PAPA) are caused by mutations in genes encoding proteins that directly or

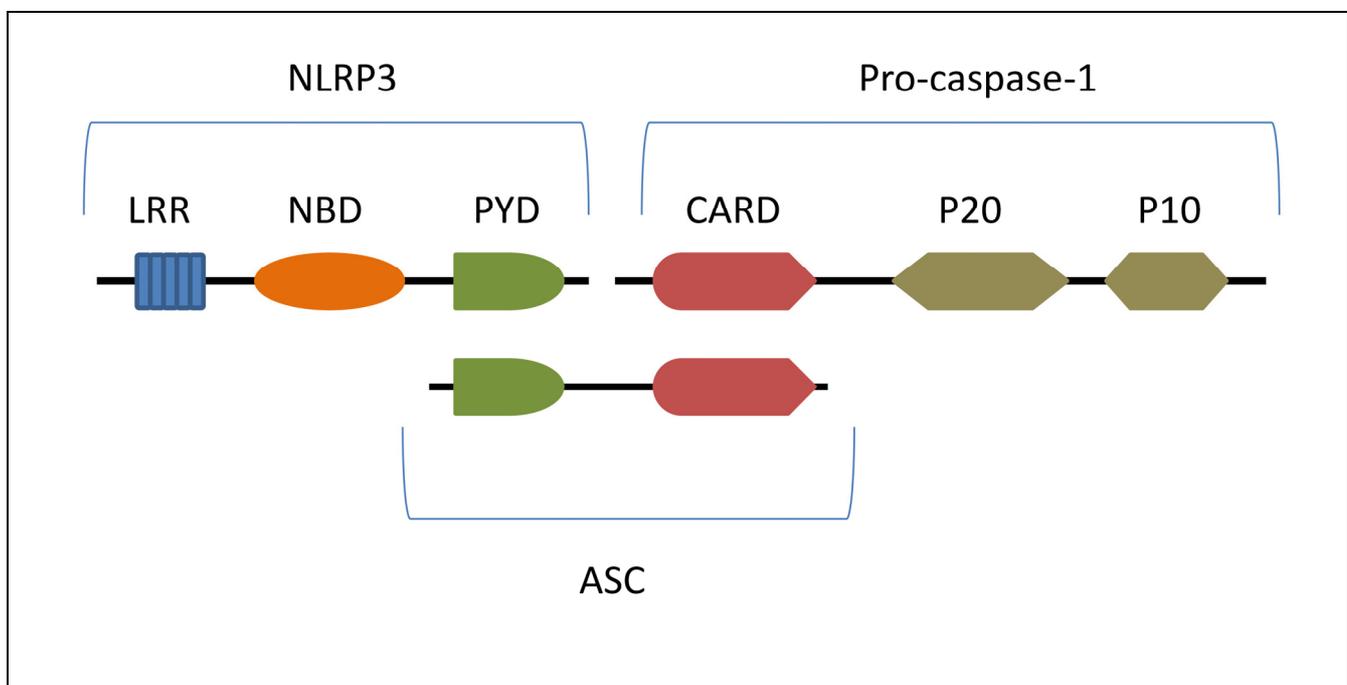


indirectly correlate NLRP3<sup>8)</sup>.

### Cryopyrin-associated periodic syndrome (CAPS) or cryopyrinopathies

The cryopyrin-associated periodic syndrome spectrum, which encompasses FCAS (Familial cold autoinflammatory syndrome), MWS (Muckle-Wells syndrome), and NOMID/CINCA syndrome (Neonatal onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, and articular syndrome), is caused by mutations in the cold induced autoinflammatory syndrome 1 (CIAS1) gene, first identified in 2001<sup>31)</sup>. CIAS1 codes for the protein cryopyrin, also known as NLRP3 or PYPAF1<sup>32)</sup>. We use the term NLRP3 thereafter. The cryopyrinopa-

thies are transmitted in an autosomal dominant pattern. The NLRP3 gene is located on chromosome 1q44 and has 9 exons. Roughly 85% of NLRP3 mutations occur in exon 3<sup>29,33)</sup>. Clinical manifestations vary among the three cryopyrinopathies, but several common features are often found, such as fever, pseudourticarial rash, joint involvement, and profoundly elevated inflammatory markers<sup>32)</sup>. The most consistent finding across the CAPS spectrum is a migratory, maculopapular, urticaria-like, and usually nonpruritic rash. Skin biopsy reveals polymorphonuclear perivascular infiltration of the dermis, which contrasts with the biopsy findings of classical urticaria. The unique features of each of the cryopyrinopathies are described below<sup>34)</sup>.



**Figure 2 Schematic structure of NLRP3 inflammasome**

Activation leads to the binding of NLRP3 with ACS through PYD-PYD interaction, resulting in recruiting of pro-caspase-1 via CARD-CARD interaction.

LRR: leucine-rich –repeat ; NBD:nucleotide-binding domain

PYD:pyrin domain; CARD:caspaseactivating and recruitment domain

#### a) Familial cold autoinflammatory syndrome (FCAS)

FCAS, also known as familial cold urticaria, is at the benign end of the CAPS spectrum, and has the most favorable prognosis of all the cryopyrinopathies<sup>32,35)</sup>. FCAS is characterized by episodes of low-grade fever, polyarthralgia, and nonpruritic pseudourticarial rash appearing 1-2 hours after cold exposure (range:

30 min to 6 h) and persisting for approximately 12 hours<sup>37,36)</sup>. Other commonly reported symptoms include conjunctivitis, profuse sweating, dizziness, headache, nausea, and extreme thirst. Symptoms are most intense in young adults, but may begin as early as childhood. Less commonly, the syndrome may present as recurrent fever, mild arthralgia, inflammatory cardiomyopathy, nephropathy, and thyroiditis, with no skin involvement. Secondary amyloidosis is



the main cause of death, occurring in up to 2% of cases<sup>37</sup>). Treatment includes prevention of cold exposure and, in more severe cases, anakinra. A recent study of rilonacept, a long-acting soluble receptor that binds IL-1, found good efficacy and safety in 44 patients with FCAS. NSAIDs and corticosteroids are variably effective, and antihistamines are not effective at all<sup>38</sup>).

### **b) Muckle-Wells syndrome (MWS)**

In 1962, Muckle & Wells described a familial syndrome of urticaria, deafness, and amyloidosis affecting nine individuals<sup>39</sup>). The symptoms of MWS arise in childhood, as an urticaria-like rash with low-grade fever and arthralgia. Recurring episodes of arthritis and conjunctivitis may also occur. The most characteristic manifestation of MWS is sensorineural hearing loss, which is due to chronic inflammation of the organ of Corti with cochlear nerve atrophy<sup>40</sup>). Less common findings include oral and genital ulcers, cystinuria, ichthyosis, recurrent abdominal pain, and microscopic hematuria. Secondary amyloidosis is common, and may occur in 1/3 to 1/4 of patients. A finding of NLRP3 mutation confirms the diagnosis. Other laboratory findings include thrombocytopenia, anemia, and increased levels of acute-phase reactants<sup>41</sup>). As in the other cryopyrinopathies, IL-1 receptor inhibition with anakinra can reverse the clinical manifestations of MWS, including hearing loss.

### **c) Neonatal onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, and articular syndrome (NOMID or CINCA syndrome)**

NOMID, or CINCA syndrome, is the most severe phenotype of the cryopyrinopathy spectrum, and was first described by Prieur & Griscelli in 1981<sup>42</sup>). The disease is characterized by a triad of rash, chronic aseptic meningitis, and arthropathy. Clinical manifestations arise in the first weeks of life; the cutaneous lesions often appear within hours of birth<sup>43</sup>). Inflammatory symptoms (such as fever) are practically continuous, with occasional flares, and affected children have severe growth retardation.

Skin lesions are found in nearly 100% of cases. CNS involvement is the second most common feature, typically presenting as chronic aseptic meningitis with leukocyte infiltration of the cerebrospinal fluid, which leads to a broad range of symptoms including chronic irritability, headaches, seizures, tran-

sient hemiplegia, and lower limb spasticity. If left untreated, approximately 80% of patients will develop sensorineural hearing loss and ocular disease, such as conjunctivitis, anterior and posterior uveitis, papilledema, and optic nerve atrophy with loss of vision<sup>44</sup>). Other findings include developmental delay and mental retardation. Patients with NOMID/CINCA syndrome have a typical facial appearance, characterized by frontal bossing, macrocrania, and saddle nose. The musculoskeletal changes of NOMID/CINCA syndrome can range from asymptomatic arthritis to deforming arthropathy. Most patients show inflammatory changes of the long-bone epiphyses and metaphyses, with abnormal epiphyseal calcification and cartilage overgrowth, leading to shortened limbs and joint deformities. Premature ossification of the patella, with symmetrical patellar overgrowth, is a characteristic finding<sup>43</sup>). The typical arthropathy of NOMID is found in 50% of patients<sup>43</sup>).

Nonspecific laboratory changes are as in other autoinflammatory syndromes, and may include anemia, thrombocytosis, moderately increased white blood cell counts, and increased inflammatory markers, such as ESR and CRP levels. The diagnosis of NOMID/CINCA syndrome relies on adequate clinical suspicion and confirmatory genetic testing. However, only 50% of patients with a characteristic presentation of NOMID/CINCA syndrome have NLRP3 mutations, which suggests that other yet-unknown genes may also be involved in its pathophysiology.

Without early identification and treatment, the prognosis for patients with NOMID/CINCA syndrome is guarded. In addition to deforming articular involvement and neurologic sequelae, the disease carries a high risk of secondary amyloidosis in the few patients who live to adulthood. Anakinra, an IL-1 receptor antagonist, is currently the drug of choice for treatment of NOMID and has been widely used in this indication, providing significant improvement in all clinical manifestations of the disease and, consequently, patient quality of life<sup>43</sup>). Corticosteroids and NSAIDs can provide symptomatic relief, but have no effect on articular or neurologic involvement.

Recently, canakinumab, which targets selectively human IL-1 $\beta$  with high affinity and prevents the cytokine from interaction to its receptor, is reported to effectively block the inflammatory response in CAPS. In all studies performed, canakinumab



showed a rapid improvement of symptoms of CAPS and a complete clinical response was achieved in most patients. Inflammatory markers such as C-reactive protein and serum amyloid-A protein were reduced to normal levels within few days. In comparison to other IL-1 blockers, canakinumab provides a longer plasma half-life and less injection site reactions<sup>45, 46</sup>.

### **Mevalonate kinase deficiency (MKD) or hyper-IgD and periodic fever syndrome (HIDS)**

HIDS follows an autosomal recessive pattern of inheritance, and is most often diagnosed in Northeastern Europe. The disease is caused by mutations in the MVK (mevalonate kinase) gene, which was discovered in 1999<sup>47</sup>. MVK, which has 11 exons and is located on the long arm of chromosome 12 (locus 12q24), codes for mevalonate kinase (MK), a 396-amino acid-long enzyme. Most patients have a combination of two mutations, one of which is very often V377I. HIDS-associated mutations lead to a major reduction in MK activity (1 to 10% of normal levels), whereas mutations that completely eliminate MK function lead to a condition known as mevalonic aciduria (MA)<sup>48, 49</sup>. MA is a rare disease characterized by periodic fever with severe CNS involvement, mental retardation, ataxia, myopathy, poor growth, and early death.

MK plays an essential role in the isoprenoid and cholesterol synthesis pathways. It catalyzes the conversion of mevalonic acid to mevalonate 5-phosphate during the synthesis of molecules such as cholesterol, vitamin D, biliary salts, corticosteroids, and non-steroidal isoprenoid compounds. During cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (the enzyme inhibited by statins) converts HMG-CoA to mevalonate, which is then phosphorylated to mevalonate phosphate. Mutations in the MVK gene block this pathway, preventing the conversion of mevalonate to mevalonate phosphate. The absence of a negative feedback loop, which is naturally provided by the presence of the end products of synthesis, leads to increased HMG-CoA reductase activity, consequently increasing serum, tissue, and urine levels of mevalonic acid. *In vitro* studies have shown that reduced synthesis of isoprenoids is associated with increased production of IL-1 $\beta$ <sup>50</sup>. Another recent *in vitro* study showed that MK inhibition leads to increased secretion of IL-1 $\beta$  due to activa-

tion of caspase-1, the enzyme that catalyzes formation of active IL-1 $\beta$  from its precursor<sup>51</sup>. High levels of immunoglobulin D (IgD) are characteristic of HIDS, but are apparently not associated with the severity of pathophysiology of the condition<sup>52</sup>.

In MKD, febrile attacks occur more frequently in the first year of life, lasting 3 to 7 days and recurring every 4 to 6 weeks. However, the time elapsed between episodes can vary from patient to patient and even in a single individual. Febrile episodes recur for years, most frequently in childhood and adolescence, but months to years can go by between flares. Episodes may be triggered by immunization, trauma, surgery, or stress, and are characterized by high fever preceded by chills. Lymphadenopathy is extremely common. It is usually cervical, bilateral, and painful. Abdominal pain is also a frequent symptom, and may be accompanied by vomiting and/or diarrhea. Patients will also frequently report headache, and splenomegaly and hepatomegaly are common. Polyarthralgia and non-erosive arthritis of the large joints, particularly of the knees and ankles are also common. Arthritis is usually polyarticular and symmetric. Most patients have diffuse cutaneous lesions, which may consist of erythematous maculopapular rash, urticaria-like rash, erythematous nodules, petechiae, or purpura. Febrile episodes may be accompanied by sudden increase in acute phase reactant levels, including neutrophilic leukocytosis and elevated ESR, CRP, and SAA. Measurement of urinary mevalonate levels during attacks may be useful, particularly in patients with normal IgD levels.

IgD levels are persistently high ( $\geq 100$  U/mL) in most patients. Nonetheless, IgD levels may be within normal limits in some HIDS patients, especially children under the age of 3<sup>52</sup>. Furthermore, the finding of high IgD levels is not specific for HIDS, as it occurs in other inflammatory diseases, such as FMF and TRAPS.

The diagnosis of MKD is confirmed by a finding of MVK mutations. However, the presence of a clinical phenotype consistent with the disease in conjunction with high serum IgD and urinary mevalonate levels may suggest the diagnosis.

Most of the usual treatments, such as NSAIDs, corticosteroids, IVIG, colchicine, and thalidomide, are ineffective in HIDS. The involvement of MK in the cholesterol synthesis pathway has encouraged the introduction of statins in the management of MKD; the efficacy of simvastatin, an HMG-CoA reductase inhibitor, has been demonstrated in 5/6 of MKD pa-



tients<sup>53</sup>). Use of etanercept and anakinra in refractory cases has also been reported<sup>54, 55, 56</sup>. Recently two patients with MVA have been treated successfully with stem cell transplantation<sup>57</sup>.

### **Pyogenic aseptic arthritis, pyoderma gangrenosum, and acne syndrome (PAPA syndrome)**

PAPA syndrome is an autosomal dominant disease characterized by sterile, deforming arthritis, skin ulcers (pyoderma gangrenosum), and severe cystic acne. Unlike other autoinflammatory syndromes, PAPA does not have fever as its most prominent symptom.

PAPA syndrome is caused by mutations in the gene that codes proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1), and only five associated mutations have been reported thus far. PSTPIP1 is a 416-amino acid-long protein expressed mostly in neutrophils. Mutations in PSTPIP1 are believed to lead to hyperphosphorylation of the protein, which could increase the potency of its binding to pyrin, with subsequent activation of IL-1 $\beta$  production, as seen in FMF<sup>58</sup>.

### **Deficiency of interleukin-1-receptor antagonist (DIRA)**

A new autosomal recessive AIS, caused by mutations in the IL1RN gene, which codes for interleukin-1 receptor antagonist (IL1Ra), was reported recently<sup>59</sup>. The syndrome, which was described in 10 patients, was given the name “deficiency of interleukin-1 receptor antagonist” (DIRA) and is characterized by early onset of symptoms, most frequently in the neonatal period.

Patients with DIRA present with pustulosis, multifocal aseptic osteomyelitis, and markedly elevated ESR and CRP levels. Skin involvement may range from sparse pustules to generalized pustular dermatitis or ichthyosiform lesions. Skin biopsy may reveal neutrophilic infiltration of the epidermis and dermis, pustules in the stratum corneum, acanthosis, and hyperkeratosis. All patients described in the report had osteomyelitis, characterized by pain with movement and periarticular swelling; the most frequent radiological findings were widening of the costal arches, periosteal elevation along long bones, and multifocal osteolytic lesions.

As in the other pyogenic autoinflammatory syndromes (PAPA and Majeed syndrome), fever is not a striking feature of DIRA, and was not present in any

of the patients described. Two of the 10 patients had interstitial lung disease, and three died before therapy could be attempted (at 2 months, 21 months, and 9 years of age respectively).

The treatment of choice is recombinant IL-1RA (anakinra), which produces a dramatic response in skin and bone symptoms and in the quality of life of patients with DIRA.

### **Pitfall for diagnosis of NOMID/CINCA syndrome**

Recent genetic studies revealed that CAPS patients usually carry heterozygous mutations in the NLRP3 coding region (mutation positive patients)<sup>60, 61, 62, 63, 64, 65</sup>. Although they exhibit no recognizable differences in clinical symptoms or in their response to treatment, approximately half of CINCA syndrome patients lack detectable mutations in NLRP3, as assessed by conventional genomic sequencing (mutation-negative patients)<sup>32, 60, 61, 62, 66, 67</sup>, indicating the existence of genetic heterogeneity among CAPS patients. Recently, we reported a patient with CINCA syndrome exhibiting mosaicism of a disease-associated mutation of NLRP3<sup>68</sup>. This case suggested that some mutation-negative CAPS patients might have mosaicism of the NLRP3 mutation; however, the contribution of NLRP3 mosaicism to disease is controversial. Aksentijevich et al claimed that NLRP3 mosaicism is a rare event in mutation-negative patients, based on their analysis of 14 patients in which NLRP3 mosaicism was not identified, even with careful bidirectional sequencing<sup>32, 68</sup>.

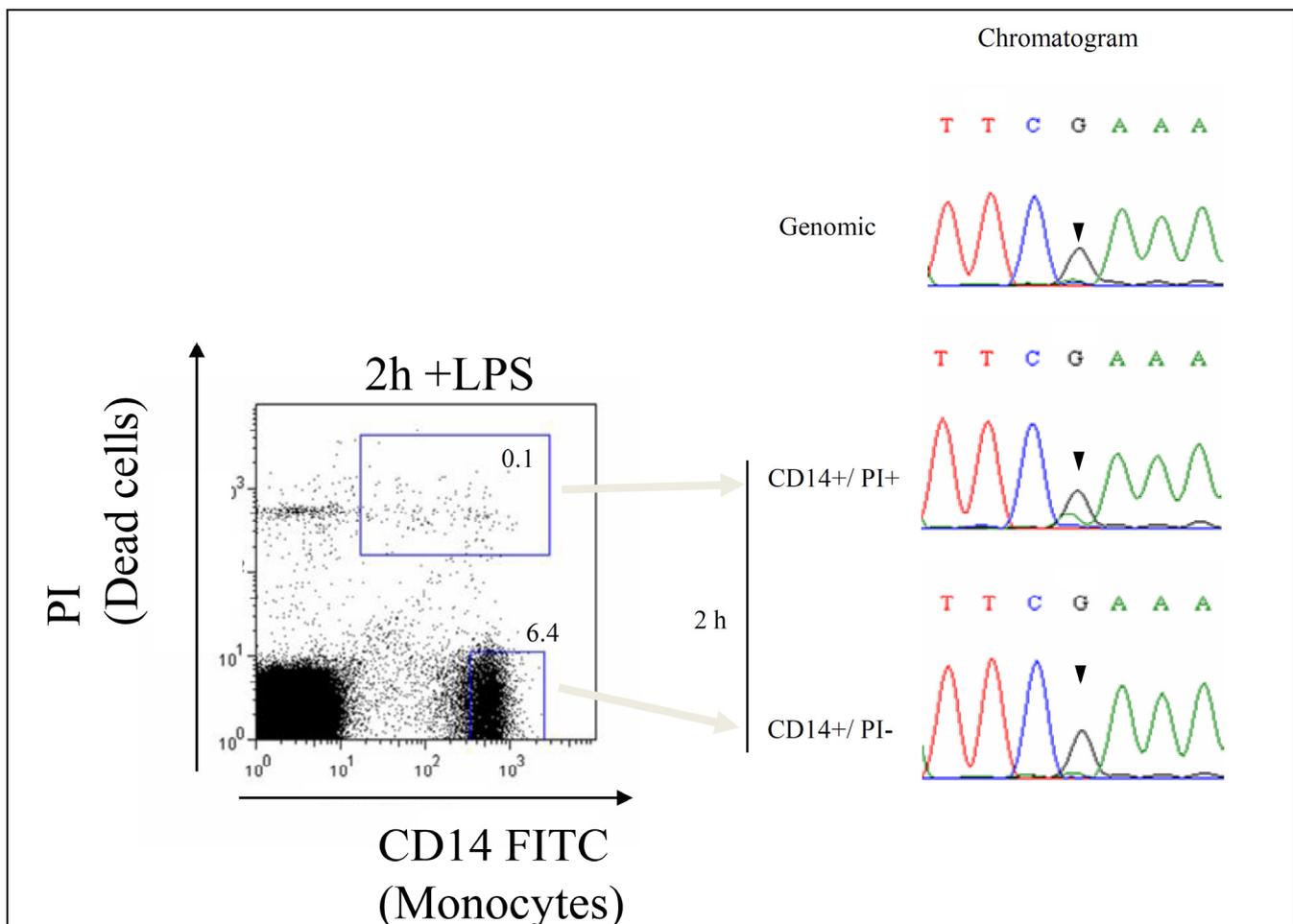
Somatic mosaicism has been reported in a number of autosomal dominant monogenic diseases<sup>69</sup>. Diagnosis of mosaicism by conventional genomic sequencing using the dideoxy termination method is often difficult, because the overlapping chromatogram of the mutant is easily missed when the frequency of a mutant allele is less than 20% to 30%. Heteroduplex-based methods or subcloning-based analysis of mutant alleles enable one to detect such low-level mosaicism; however, these methods are resource intensive, and cannot distinguish whether the detected mutation is disease-causing or simply a nonfunctional single nucleotide polymorphism (SNP). An alternative approach involves the isolation of mutant cells using functional analyses based on their characteristic biologic features, and then determining the DNA sequence of the isolated cells. Based on these backgrounds, we set out to identify specific biologic features of NLRP3-mutant cells compared



with nonmutated cells, in an effort to specifically isolate NLRP3-mutated cells from mutation-negative patients<sup>70</sup>.

Disease-associated NLRP3 mutations induce ASC-dependent NF- $\kappa$ B activation in some systems, and we reported that they also induce necrotic cell death in the human monocytic cell line THP-1, which is a novel function of NLRP3<sup>68</sup>. Based on these backgrounds, we explored whether NLRP3-mutant cells have specific biologic features, using monocytes

from mutation-positive patients, and found that NLRP3-mutant monocytes rapidly underwent necrosis-like cell death after treatment with lipopolysaccharide (LPS) to induce NLRP3 expression. This unique phenotype of NLRP3 mutant cells enabled us to differentiate NLRP3-mutated cells and nonmutated cells in 3 of 4 mutation-negative CAPS patients, and we were able to successfully demonstrate that these 3 patients had mutations of NLRP3 as latent mosaicism (Fig. 3)<sup>71,72</sup>.



**Fig. 3 Enrichment of dying monocytes in mutation-negative patient revealed latent overlapping peaks**

PBMCs from mutation-negative CAPS patient were cultured with or without LPS (10 ng/mL) for 24 hours. CD14-positive and -negative cells were sorted, and DNA was extracted and sequenced for analysis of NLRP3.

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