
Autoinflammatory syndromes and infections: pathogenetic and clinical implications

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Abbreviations:

NLR:	NOD-like receptors
NOD:	Nucleotide oligomerization domain
NBD:	Nucleotide binding domain
NALP:	NACHT- LRR- and pyrin domain containing
ASC:	Apoptosis associated speck-like protein containing a CARD
IPAF:	IL-1 β converting enzyme protease activating factor
T3SS/T4SS:	Type III or type IV secretion system
NAIP:	Neuronal apoptosis inhibitor protein
CIITA:	Major histocompatibility complex- Class II trans activator
NACHT:	Domain present in NAIP, CIITA, HET E and TP1
NAD:	NACHT-associated domain
CARD:	Caspase recruitment domain
PYD:	Pyrin domain
BIR:	Baculovirus IAP repeat
LRR:	Leucine-rich repeat
PAMP(s):	Pathogen-associated molecular pattern(s);
DAMP(s):	Danger-associated molecular pattern(s);
CIAS1:	Cold-induced autoinflammatory syndrome 1;
MSU:	Monosodium urate;
CPPD:	Calcium pyrophosphate dihydrate;
AOSD:	Adult-onset Still's disease;
ESR:	Erythrocyte sedimentation rate;
CRP:	C-reactive protein;
SAA:	Serum amyloid A;
DMARD(s):	Disease-modifying antirheumatic drug(s);
MTX:	Methotrexate;
BS:	Blau Syndrome;
IFN:	Interferon;
NF- κ B:	Nuclear factor- κ B;
FMF:	Familial Mediterranean fever;
TRAPS:	TNF- receptor associated periodic syndrome;
MKD:	Mevalonate kinase deficiencies;
TNFRSF1A:	TNF super family receptor 1A;
HMGR:	Hydroxymethylglutaryl-coenzyme A reductase;
SREBP:	Sterol regulatory element binding proteins;
PBMC:	Peripheral blood mononuclear cells.

ABSTRACT

The autoinflammatory syndromes are a group of disorders characterized by recurrent episodes of seemingly unprovoked inflammation without significant levels of autoantibodies and antigen specific T cells. Although a direct association between defective innate immune responses to bacterial components and these diseases has not been formally established, much ongoing research is aimed towards confirmation of that hypothesis.

This article will review recent advances in the study of a subset of NOD-like receptors (NLRs), which control the activation of caspase-1 through the assembly of a large protein complex called inflammasome. Moreover, we will review recent progresses in understanding of a range of autoinflammatory conditions in humans.

Introduction

Immune system and autoinflammatory disease

The immune system is classically subdivided into two parts, the innate and the acquired immunity.

In autoimmune diseases autoantibodies or autoreactive T-cells, both effectors of acquired immune system, induce tissue inflammation (1-12). Therefore the acquired immunity is the part of immune system mostly involved in auto-immunity.

In autoinflammatory disease the innate immune system causes inflammation for unknown reasons, because the body spontaneously produces substances that cause inflammation without the formation of autoantibodies or any other immunological stimulus (13).

The term "autoinflammatory" was coined by Daniel L. Kastner, and has been applied to a group of distinct hereditary disorders (Table I) characterized by unexplained episodes of fever and severe inflammation, involving most commonly skin and joints.

Competing interests: none declared.

Table I. Principal autoinflammatory disorders.

Familial Mediterranean fever (FMF)
Hyper-IgD syndrome (HIDS)
Pyogenic arthritis, pyoderma gangrenosum, acne (PAPA)
Periodic fever aphthous stomatitis and adenitis (PFAPA)
Muckle-Wells syndrome (MWS)
Familial cold autoinflammatory syndrome (FCAS)
Neonatal-onset multisystem inflammatory disease (NOMID)
TNF receptor-associated periodic syndrome (TRAPS)
Adult-onset Still's disease (AOSD)
Blau syndrome
Behçet's syndrome
Crohn's disease
Gout

Significant advances in understanding of the molecular basis of inflammatory mechanisms have occurred in the last 10 years, with the identification of the genetic basis of some autoinflammatory syndromes; here we review some biochemical evidences underlining the role of inflammasome and inflammatory caspases in innate immunity against pathogens and their involvement in autoinflammatory syndromes, finally we discuss some of these disorders.

Inflammasome: a sensor of immune danger signals

The mammalian NOD-like receptor (NLR) family of intracellular proteins contains 22 members including 14 NALP members, 5 members of the NOD subfamily, IPAF, NAIP and CIITA. The NLRs are characterized by a unique nucleotide-binding domain called NACHT, which is located at the center of the molecule between an N-terminal protein-binding domain (CARD (caspase-recruitment domain), PYD (pyrin domain) or BIR (Baculovirus IAP repeat) and a C-terminal LRR (Leucine-rich repeat) domain.

The NLRs have been implicated in sensing pathogen-associated molecular patterns (PAMPs) as well as cellular danger signals (danger-associated

molecular patterns; DAMPs). The importance of NLRs in these innate immune functions can be demonstrated by the finding that mutations within a number of NLR genes have been associated with autoimmune and autoinflammatory disorders in humans. Mutations in NOD2 have been associated with Crohn's disease and Blau syndrome. Mutations within the *NALP3/CIAS1* gene are responsible for the autoinflammatory syndromes, Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and neonatal-onset multisystem inflammatory disease. Most recently sequence variants in the *NALP1* gene have been linked to autoimmune and autoinflammatory diseases associated with vitiligo (14). It is of interest that human disease associated with NLR mutations identified to date are predominantly autoimmune or autoinflammatory in nature as opposed to causing increased susceptibility to bacterial or viral infections. However, it remains a possibility that inappropriate responses to either pathogenic or commensal microbes may serve as a trigger for some of these disorders.

Activation of the NALP3 inflammasome by danger signals

A number of NLR molecules (NALP1, NALP3, and IPAF) can activate caspase-1 within a multiprotein complex called the inflammasome (Fig. 1). *In vitro* studies have also demonstrated that NALP2, NALP6, NALP7, and NALP12 can form caspase-1 activating inflammasomes, however studies to demonstrate the physiologic roles of these molecules is still pending. The components involved in the formation of the inflammasome have been recently reviewed in detail (15, 16).

Numerous DAMPs have been identified that can activate the caspase-1 inflammasome. The best studied of these DAMPs is ATP. The stimulation of cells with high concentrations of ATP is thought to mimic the rapid release of ATP from activated platelets, neurons, antigen-stimulated T cells and injured cells. The effect of ATP is mediated by an ionotropic ATP receptor, P2X₇, which causes a rapid K⁺ efflux from the cytosol upon activation. Activation of caspase-1

by ATP is dependent on NALP3 and the adaptor molecule ASC (16). Similar to ATP, a number of bacterial pore-forming toxins have been shown to activate caspase-1 in a manner that is dependent on a K⁺ efflux, NALP3 and ASC. These toxins include nigericin (*Streptomyces hygroscopicus*), maitotoxin (*Gambierdiscus toxicus*), aerolysin (*Aeromonas hydrophila*) and listeriolysin O (*Listeria monocytogenes*) (15, 16). Recent studies have identified that pannexin-1, a membrane protein that can act as a non-selective pore, is required for caspase-1 activation in response to ATP, nigericin, and maitotoxin. It is unclear how Pannexin-1 acts to activate the inflammasome, but Kanneganti *et al.* have proposed that the Pannexin-1 pore may allow PAMPs to gain entry to the cytosol and activate NALP3 (17).

Monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals, the causative agents of gout and pseudogout respectively, have also been shown to activate caspase-1 in a NALP3 dependent manner (16). In addition to its role in gout, uric acid is a major component released into the extracellular milieu by necrotic cells. Recognition of uric acid following cell death may be an additional way in which NALP3 detects endogenous danger signals. It is unclear if activation of the NALP3 inflammasome by MSU and CPPD is induced by host cell membrane damage, in a manner similar to the bacterial pore-forming toxins or the Pannexin-1 pore.

Skin irritants such as 2, 4, 6-trinitrochlorobenzene (TNCl, TNP-Cl) and 2, 4-dinitrofluorobenzene (DNFB) have been shown to activate the NALP3 inflammasome and mice deficient in NALP3 or ASC display impaired contact hypersensitivity reactions to TNCl (16). In addition, damage by UVB-irradiation has also been shown to activate the NALP3 inflammasome in keratinocytes (18).

Activation of the IPAF inflammasome by type III and IV secretion systems

A number of gram-negative organisms have been identified that are capable of activating the inflammasome. Caspase-1-mediated cell death and IL-1 β secretion caused by infection with

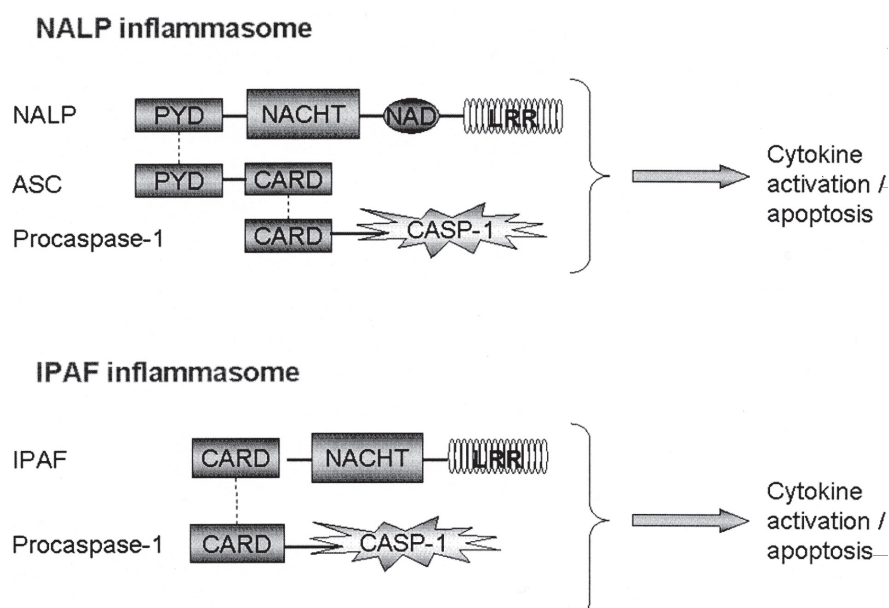


Fig. 1. Structural organization of NALP and IPAF inflammasomes.

NALP inflammasome is formed by a NALP, the adaptor ASC and procaspase-1. PYD-PYD and CARD-CARD interactions are important for the recruitment and activation of either the adaptor ASC or the inflammatory procaspases.

IPAF recruits procaspase-1 directly via CARD-CARD interactions.

IPAF: NACHT- LRR- and pyrin domain containing; PYD: pyrin domain; NACHT: domain present in NAIP, CIITA, HET E and TP1; NAD: NACHT-associated domain; LRR: leucine-rich repeat; CARD: caspase recruitment domain; ASC: Apoptosis associated speck-like protein containing a CARD; CASP-1: caspase 1; IPAF: IL-1 β converting enzyme protease activating factor.

Salmonella typhimurium, *Shigella flexneri*, *Legionella pneumophila*, and *Pseudomonas aeruginosa* are dependent on IPAF (15, 19-21). In contrast, *Francisella tularensis* activates an inflammasome that is not dependent on NALP3 or IPAF but does require ASC (22). *F. tularensis* does require signaling through the type I IFN receptor for efficient inflammasome activation (22). It remains to be seen if the type I IFN receptor will play a central role for activation of the inflammasome by other pathogens. The role for ASC in IPAF-mediated caspase-1 activation is unclear. Although IPAF can interact directly with pro-caspase-1, ASC-deficient macrophages have defective caspase-1 activation following infection with either *S. typhimurium* or *P. aeruginosa* suggesting that ASC does play an important role in the IPAF inflammasome.

Recent studies have begun to provide insight into how the IPAF-inflammasome is activated. *S. typhimurium* and *L. pneumophila* strains deficient in flagellin were found to be defective in their ability to activate caspase-1 (23). Purified flagellin delivered to the

macrophage cytosol by transfection was also capable of activating caspase-1. These findings led to the hypothesis that cytosolic flagellin is inadvertently delivered to the cytosol through either the type III (T3SS) or type IV (T4SS) secretion system which results in the activation of caspase-1. However, the direct activation of IPAF by cytosolic flagellin remains controversial. Both non-flagellated *S. flexneri* and a *P. aeruginosa* mutant deficient in flagellin are capable of activating caspase-1 in an IPAF-dependent manner suggesting that flagellin is not required for IPAF activation (19-20). In addition, at high multiplicity of infection-flagellin-deficient *S. typhimurium* strains are still capable of inducing macrophage secretion of IL-1 β (23).

S. typhimurium, *S. flexneri*, and *P. aeruginosa* all require an intact T3SS to activate caspase-1. *L. pneumophila*-induced caspase-1 is dependent on the Dot-Icm T4SS which is structurally unrelated but functionally similar to the T3SS. Since flagellin alone is not required to activate the IPAF-inflammasome, it is possible that rather than

detecting a specific pathogen-derived molecule, IPAF detects membrane damage induced by either T3SS or T4SS. Although rapid progress has been made in understanding how the inflammasome is activated it remains unclear if the activating ligands for NALP3 and IPAF interact directly with these molecules or do so indirectly through the release of an endogenous ligand. Further studies to elucidate the events leading to activation of the inflammasome will be required to help further understand the physiologic function of NLRs.

Adult-onset Still's disease

Adult-onset Still's disease (AOSD) is a rare systemic inflammatory disorder, with a yearly incidence of approximately 0.16/100,000 of the population. Classically, it presents with evanescent rash, fever and articular involvement. AOSD usually occurs in people of young age between 16 and 35 years old, without a sex bias (24).

The etiology of AOSD remains a mystery, and a complex interplay of genetic, environmental, and neuropsychogenic factors, most of which are not yet identified, is thought to influence the disease process. The low prevalence of the disease, its variable phenotypic expression and the absence of collaborative disease registries have all hindered the efforts to elucidate the disease pathogenesis. Multiple genetic studies of the MHC complex have failed to identify universally relevant HLA associations, and have often produced conflicting results.

Various infectious agents have been suggested to have a triggering effect upon as triggers of the pathogenetic sequence, due to temporal associations between disease onset and viral syndromes, the presence of positive serologies and, especially, the resemblance of the AOSD phenotype to various viral syndromes. Viruses implicated include parvovirus B19, rubella, echovirus 7, HHV- 6, parainfluenza, EBV, CMV, coxsackie B4, mumps, and adenovirus, while associations with Chlamydia, Yersinia, Brucella and Borrelia species have been inferred at times (25).

Immune dysregulation, favoring a Th1 over a Th2 response, has been suggested from cytokine measurements

in sera and pathological tissues of AOSD patients when compared with healthy controls. In particular, TNF- α , IL-6, IL-8, IL-1 β and IL-18 have all been found elevated in patients' sera and some correlate well with biochemical abnormalities, such as the extremely high concentrations of serum ferritin that are commonly seen in active disease (26, 27).

Typical presentation of AOSD consists of a triad of symptoms which include high spiking fever, an evanescent rash and arthritis. AOSD is frequently the indolent cause of fever of unknown origin. Typically, fever is quotidian, spiking ($> 39^{\circ}\text{C}$) and often associated with a salmon-pink color rash that may involve the proximal limbs and trunk. Joint involvement, especially of the wrists, hips, knees ankles is commonly observed and can evolve, in some patients into a chronic, destructive and ankylosing arthritis (28). Common laboratory abnormalities include neutrophilic leukocytosis, resulting from bone marrow granulocyte hyperplasia, anemia of chronic disease, transaminasemia, and elevated non-specific serum inflammatory markers, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum amyloid A.

Diagnosis of AOSD remains clinical and often necessitates the exclusion of infectious, neoplastic and other autoimmune diseases (29). Multiple sets of diagnostic and classification criteria have been developed, with Yamaguchi's criteria being the most widely used (30). Although not diagnostic, some laboratory abnormalities may be helpful in diagnosis and could be potential biomarkers (31). Serum ferritin levels higher than five times (approximately 1000ng/ml) the upper limits of normal, although by no means disease-specific, may suggest the presence of the disease with an 80% sensitivity and 46% specificity. Ferritin has been proposed to be a very useful marker of disease activity as its levels correlate with clinical scores and tend to depict clinical response to treatment. Ferritin's glycosylated fraction, consistently found less than 20% in AOSD, may become a more specific diagnostic test, if it becomes readily available (32, 33).

Corticosteroid use came into the therapeutic forefront due to its potent anti-inflammatory actions. Despite their success in suppressing both systemic and articular symptoms, they appear to be ineffective in modifying progressive joint destruction in the chronic articular form. DMARD use during the course of treatment of AOSD often becomes a necessity in refractory cases where corticosteroids have failed, as steroid sparing agents, or in an attempt to prevent chronic destructive arthritis (34). Methotrexate (MTX) is the most widely used DMARD, often in combination with low dose corticosteroids, in chronic cases. Apart from MTX, other DMARDs used to treat AOSD with less documented efficacy are cyclosporine A, hydrochloroquine, gold, penicillamin, azathioprine, leflunomide, cyclophosphamide and tacrolimus, while sulfasalazine lacks efficacy and has been associated with severe adverse effects. Results from the usage of biologic agents, initially in cases refractory to corticosteroids and DMARDs, have shown promise, signaling a new era in disease therapeutics and a paradigm shift. TNF- α inhibitors were initially utilized, although there may be less convincing evidence regarding TNF- α involvement, when compared to other cytokines, such as IL-6 and members of the IL-1 family (IL-1 β , IL-18). Multiple case reports and small series suggested that infliximab, etanercept, and, in one instance, adalimumab, were effective in refractory disease. The largest published observational study of 20 patients with refractory AOSD, suggested that when the disease responded to anti-TNF agents, clinical improvement occurred rapidly, within the 2-6 weeks of treatment. Most of the cases showed partial response, with complete remission observed in 5/20 cases, and switching between etanercept and infliximab added little in terms of efficacy (35). With the clinical importance of the NALP3 inflammasome recently demonstrated in AOSD, IL-1 inhibition has emerged as a prominent therapeutic strategy. Anakinra, a recombinant IL-1 receptor antagonist given subcutaneously at a daily dose of 100 mg, has been reported in at least 23 published cases to achieve a

rapid resolution of symptoms and normalization of laboratory values, including white blood cell counts, serum ferritin, and CRP levels (36). Remission has been achieved even within hours, while discontinuation of anakinra resulted in rapid clinical deterioration and necessitated prompt reinstitution of the agent. Tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody in development for RA, has demonstrated therapeutic efficacy in AOSD in Japan, where it is currently available, and may become in the future a therapeutic alternative (37). It should be noted that biologic agents, however efficacious, have been associated with rare, although severe, side effects in AOSD and their use should be restricted to experienced users and, ideally, their efficacy and safety should be confirmed in controlled studies (38).

AOSD is a rare, systemic, inflammatory disease of unknown etiology. Advances in our understanding of its pathophysiology and cytokine biology have opened the window of opportunity for rational targeted treatment with currently available and upcoming biologic agents.

Granulomatous autoinflammatory syndromes

Granulomatous inflammatory reactions are frequently observed in children. However, those involving joints are rarely found and mostly associated with a common genetic trait identified in the CARD15/NOD2, and a phenotype characterised by a multisystemic disorder. The most representative of these syndromes are Crohn's disease, early-onset sarcoidosis and Blau syndrome (BS), all associated with different frequency to CARD15/NOD2 gene mutations. Miceli-Richard *et al.* first discovered in 2001 that the gene responsible for BS was in the caspase recruitment domain (CARD15), also named NOD2 (39). They identified three missense mutations (R334Q, R334W and L469F) in the nucleotide-binding domain (NBD) of CARD15/NOD2 in four French and German families with BS. Noteworthy, although missense mutations of BS and Crohn's disease are in the same gene CARD15/NOD2, the disease associated polymorphisms of

Crohn's disease are located in the leucine-rich repeat (LRR) regions of the protein, a domain that is involved in the recognition of bacterial lipopolysaccharides. Probably, this localisation may be in keeping with bowel involvement characteristic of this disease and never found in BS. The other disease candidate to be part of the group of granulomatous autoinflammatory syndromes is the early-onset sarcoidosis, which seems to be a sporadic counterpart of BS, and may share with this disease the same genotype *CARD15/NOD2* and a phenotype mainly featuring the classic triad of arthritis, dermatitis and uveitis (40). However, the presence of other visceral involvements, the absence of familial forms, and the different outcome have induced most authors to propose that the term of granulomatous auto-inflammatory syndrome should be only deserved to BS.

BS is a rare familial disease characterized by arthritis with sometimes campodactily, uveitis, skin rash and granulomatous inflammation (39). Blau first described a family with eleven members over four generations having granulomatous disease of the skin, eyes, and joints. Ten had arthritis; two had skin, eye, and joint involvement; one had skin and joint disease, and one had iritis only (41). Blau observed that the disease was transmitted as an autosomal dominant trait and resembled only partially sarcoidosis, and therefore it probably represented a new syndrome. In addition to three missense mutations (R334Q, R334W and L469F) previously identified by Miceli-Richard, we recently found a new *CARD 15* mutation (E383K) in a family followed-up by us for 25 years (42). To our knowledge, this is the only family affected with BS in Italy, although two other sporadic cases have been also observed. In our family, both the proband and her daughter were affected first with a papulonodular skin eruption and then with mild arthritis involving hands and feet. The proband, but not the daughter, complained of severe chronic bilateral uveitis, followed by glaucoma and, a few years later, cataracts. Histological examination of skin biopsy performed in both subjects and joint biopsy executed only in the

daughter, shows non-caseating granulomas with multinucleated giant cells, which at the electron microscopy revealing "comma-shaped bodies" in epithelioid cells, which is thought to be a marker for BS. The disease is at present well controlled with low doses of prednisone in the mother and NSAIDs with the addition of low doses of prednisone, when necessary, in the daughter.

Recently it has been suggested that pediatric granulomatous arthritis, a chronic disease resembling Blau syndrome/early-onset sarcoidosis, may also be associated with mutations in *CARD15/NOD2* gene (43). These findings indicate that the expanding clinical heterogeneity of the pediatric granulomatous diseases syndromes and the high prevalence of sporadic cases should alert clinicians to the possible genetic basis of the condition and support the inclusion of DNA analysis as a diagnostic test.

Although in comparison with Crohn's disease, the mechanism by which the gene is responsible for the pathogenesis of BS is less known (44), it has been suggested that also in BS bacteria may have a role. It has been demonstrated that *CARD15/NOD2* binds directly to a muramyl dipeptide that is common to all bacteria and this binding leads to activation of NF- κ B (45). Furthermore, it has been observed that *CARD15/NOD2* mutation of codon 334 causes a four-fold higher activation of NF- κ B, compared to wild type alleles (46). Finally, BS has recently been found to be associated with a distinctive mutation in *CARD15/NOD2*, which encodes an intracellular Toll-like receptor.

As increasingly evident for the whole group of autoinflammatory syndromes, the study of the mechanisms involved in the pathogenesis of BS may probably shed new light on the knowledge of the mechanisms of the other granulomatous inflammatory diseases.

Familial Mediterranean Fever

Familial Mediterranean fever (FMF, OMIM *249100) is an autosomal recessive disorder characterised by short, recurrent, apparently unprovoked and with spontaneous resolution attacks of fever and serositis, or erysipelas-like skin lesion (47, 48).

FMF is the most frequent periodic febrile syndrome among autoinflammatory syndromes (47). It has been calculated by M. Pras that it affects more than 100 thousands people in the world. Although it is prevalent among Turks, Armenians, Arabs and Jews (49), cases of FMF have been reported in other countries (50, 51), and in Italy too (52).

Mediterranean fever was firstly described in 1945 but the responsible gene (MEFV) was discovered in 1997 by two different study groups (French and American) (53, 54). MEFV gene is located on chromosome 16p13.3 and encodes a 781-aminoacid protein (pyrin/marenostrin), involved in the regulation of inflammation and apoptosis (55).

Pyrin is expressed in the myeloid cell lineage and participates in inflammation formation, a macromolecular complex; its dysfunction determines autoinflammatory syndromes (56-58). Pyrin consists of four domains; the most important are the N-terminal pyrin domain and the C-terminal B20.3 domain. The first one is intimately connected to protein-protein homotypic interactions, inflammation, apoptosis and immunity against tumours. The second one participates in processes of innate immunity and is codified by sequences in exon 10, whose mutations as M694V, M694I M680I, are associated with severe phenotype.

FMF attacks may be triggered by common factors such as cold exposure, emotional or physical stress, infections or menstruations (59). More than 15% of female patients experience perimenstrual attacks. It is proposed that oestrogens normally inhibit IL-6 production and mimic colchicine's effect on tubuli and adhesion molecules. During menstruations the protective effect of oestrogens disappears, leading to the acute attack.

A prodromic syndrome 12-24 hours before FMF attacks is reported (60); the premonitory symptoms include discomfort, abnormal taste sensation, dizziness, increased appetite, irritability and so on.

Laboratory tests during attacks show leukocytosis (predominantly neutrophils), increase of inflammation parameters

(ESR, CRP, SAA), microalbuminuria, which normalize in asymptomatic periods, except for patients with persistent subclinic inflammation.

Diagnosis is based on the Tel-Hashomer clinical criteria (more recently on the Montpellier criteria). Genetic analysis can confirm diagnosis but it is not mandatory.

Colchicine is still the only suitable drug to prevent the attacks and the development of amyloidosis, at a daily dose of 1-2 mg (61). Alternative experimental treatments including prazosin, TNF-antagonists (etanercept, thalidomide, infliximab), methotrexate, IL1 receptor blockade (anakinra), selective serotonin re-uptake inhibitors (SSRI), have been proposed in non-responder cases, but further studies are necessary (62-68).

We analysed demographic, clinical and genetic features of FMF patients recruited at the Periodic Fever Centre, Catholic University, Rome, over the past 8 years. We diagnosed FMF in 221/800 patients with fever of unknown origin: 111 males and 110 females, aged from 2 to 93 years, coming from Italy (74.66%) and other countries (17.2%; especially Jews). Most patients came from the South (> 60%) and the Centre of Italy (> 25%).

On the basis of Tel Hashomer Criteria, patients were subdivided into three groups: definite (68.3%), probable diagnosis (18.6%), and possible FMF (11.3%).

In 85% of patients the age of onset was under 30 years (in 40% before 10 years). FMF presented in form of recurrent attacks. A typical attack consisted of fever and serositis with a duration from 1 to 4 days and spontaneously disappearing.

Frequency of attacks varied widely: <1/month (21%), 1-2/month (36.5%), >2/month (42.13%).

Fever was present in 93.6% of patients. Abdominal involvement was the second most frequent manifestation of FMF (83%). Almost 70% of patients complained of musculoskeletal manifestations, including arthralgia (30%), arthritis (16.43%) or myalgia (9%).

Forty per cent and 34% of patients complained of chest pain (pericarditis and/or pleuritis) and *erysipelas-like*

skin lesions respectively. Few patients showed early renal damage (microalbuminuria: 4.65%; proteinuria: 4.65%; ematuria: 0.9%) and only 3.9% of cores developed amyloidosis. Among *minor* manifestations splenomegaly (13.5%) and acute orchitis (1.35%) were observed.

The genetic test was positive in 73.5% of patients (one mutation in 22.6%, two mutations in 32.21%). M694V was the most frequent mutation (24.5%), followed by E148Q (15%), M680I (13%), M694I (11.05%), V726A (5.3%). Rare mutations were observed in less than 20% of cases.

Patients were stratified according to Pras severity score in three groups: severe (23.2%), moderate (38.3%) and mild disease (38.3%). Genophenotypical correlations were similar to those of literature (69): in severe phenotypes M694V (38.7%), M680I (14.2%) and M694I (6%) were prevalent in homo- or heterozygosis, although 20% had no mutations. In mild phenotypes the most frequent mutation was E148Q (70), but in 22.2% of patients no mutations were found and in 35% M694V, M680I, M694I were detected. We hypothesise the presence of protective factors in these cases (71).

Because of diagnostic delay (13.7 years in our report), some patients underwent unnecessary surgery, without resolution of symptoms (appendectomy was the most frequently and was performed in 1/5 patients).

About 80% of patients regularly took colchicine (0.03 mg/Kg/die). About 19% did not take it: 7% never started treatment, 12% stopped treatment for gastrointestinal side effects, hepatotoxicity, inefficacy or personal decision. The response to colchicine in terms of persistence/disappearance of frequency, length and intensity of bouts was as follows: very good response in 31%, good response in 24.5%, partial response in 29%, minimal response 12.2%. Non-responder rate was less than 5% (3.5%). Therapeutic options included the addition of a non-steroidal anti-inflammatory drug during attacks, or an increase of colchicine dosage five days before menstruations. Our preliminary results suggest that etanercept is effective in non-responder patients.

TRAPS (TNF-receptor associated autoinflammatory syndrome) and MKD (mevalonate kinase deficiencies)

TRAPS (Tumor necrosis factor receptor-associated periodic syndrome) is an auto-inflammatory disorder due to autosomal dominant mutations in the p55kda TNFR, encoded by the TNF Super Family Receptor 1A gene (TNFRSF1A) (72). First descriptions were reported in 1982 in a single family of Irish/Scottish ancestry as familial Hibernian fever. Subsequent reports confirmed that this disorder could be observed in patients of virtually all ethnic origins. In typical cases TRAPS begins early in childhood and is characterized by high and remittent fever over a period of 1-4 weeks or longer, accompanied by systemic and local inflammation. Skin lesions are large, warm and tender erythemas over the trunk and extremities. They contain perivascular infiltrate of mononuclear cells. When lesions occur on the limbs or arms there may be an associated myalgia due to an underlying monocytic fasciitis. During attacks, patients exhibit elevated acute-phase reactants including erythrocyte sedimentation rate (ESR) and C reactive protein (CRP), increased neutrophil count and variable degrees of hypochromic anemia. Renal AA amyloidosis represents the most serious long-term complication, with a prevalence ranging from 14 to 25%. Central nervous demyelinating disorder has been described in few patients. More than 50 mutations have been identified in patients with TRAPS and reported online at <http://fmf.igh.cnrs.fr/infevers>. Most of them are missense mutations in the first two N-terminal cysteine-rich domains (CRD1, CRD2). Mutations resulting in substitutions of cysteine residues involved in the disulfide bonds have significant structural effect on the conformation and stability of the TNFR ectodomain (73). The first thought effect of these mutations was an alteration of activation induced shedding of TNFRSF1A, leading to defective neutralization of TNF, although it is not the case for all mutations. Moreover, TRAPS patients display a normal shedding of TNFRp75, a prevalent source of circulating soluble TNFR in normal

conditions. Data from recent literature have demonstrated that TNFRSF1A mutants may induce TRAPS by other mechanisms. Some mutations such as C43S could alter TNF- α signaling and induce apoptosis with increased or decreased NF- κ B activation (74, 75). Other abnormal behaviors of mutant TNFRSF1A consist of reduced cell surface expression and TNF binding, and increased intracellular retention in the endoplasmic reticulum (RE) (76). Phenotype-genotype correlation show that mutations resulting in cysteine substitutions are associated with severe disease course and higher risk for secondary amyloidosis. In contrast, other mutations (R92Q, P46L) present in 1–2% of healthy individuals seem to be milder and less specific. Indeed the R92Q mutation has been reported to be increased in patients with Behçet's disease and multiple sclerosis. Fever episodes in TRAPS patients usually respond to steroid treatment. However their long-term use may induce significant toxicity. The use of anti-TNF treatment is not constantly effective. IL-1 blocking agents may represent an interesting alternative in patients who do not respond to anti-TNF treatment. Mevalonate kinase deficiencies (MKD) also called MAPS for mevalonate kinase-associated periodic syndromes, are rare autosomal recessive inborn errors of cholesterol biosynthesis caused by mutations of the MK gene (MVK) compromising the biosynthesis of non-sterol isoprenes in addition to cholesterol. MKD is known to be responsible for two distinct phenotypes, mevalonic aciduria (MA) and hyper-IgD syndrome (HIDS), depending on the residual MK activity. In fact, the clinical spectrum is wider, ranging from mild forms of HIDS to lethal forms of MA, and many cases could be still undiagnosed (77). Incomplete MKD, called so far HIDS has been reported mostly in the Netherlands, France and Italy (78). The disease manifests early in infancy before the age of 3 years, in 2/3 of cases during the first year of life. The main symptoms include recurrent febrile episodes lasting 3 to 6 days, triggered by infections, immunization, physical exercise and emotional stress.

Tender cervical lymphadenopathy, headaches and gastro-intestinal symptoms (pain, vomiting) are commonly associated. Some patients develop transient arthritis, hepatosplenomegaly, oral or bipolar aphthosis, and various skin manifestations (macular rash, urticaria, erythema nodosum, purpura). During febrile crises, blood tests show leukocytosis, monocytosis, and elevated acute-phase reactants, *e.g.*, CRP, SAA protein, while the urine excretion of mevalonic acid is slightly increased (0.004–0.028 mmol/mol creatinin), but IgD levels are not constantly elevated. The clinical spectrum of MA may include febrile crises (especially in late infancy) but they diminish over time. Stunted growth, mental retardation, ataxia, retinal dystrophy and cataract are constant findings. IgD levels are constantly elevated in MA patients and they increase during febrile crises with the raise of acute-phase reactants. The urine excretion of mevalonic acid is continually increased and MK activity in lymphocytes is <1% of the healthy control. The MK enzyme catalyses the phosphorylation of MA into 5-phosphomevalonate, which is the enzyme following the HMGR (hydroxymethylglutaryl-coenzyme A reductase) in the isoprenoid/cholesterol biosynthesis pathway. MK is present in all tissues, in different amounts, and its activity decreases with temperature elevation. MK enzyme deficiency results in MA accumulation and end products shortage. Non-sterol isoprenoids include dolichols, ubiquinone and polyisoprenes side chains of heme A. They play a vital role in the prenylation of various G proteins of the Ras/Rho/Rac family. The prenylation process consists in the addition of hydrophobic lipid groups (geranyl-geranyl, farnesyl) on cytoplasmic enzymes, allowing their attachment to the plasmic membrane where their substrates are located. The needs of end products regulate the flux through the pathway to prevent either shortage or toxic accumulation. In case of cholesterol shortage, SREBP (sterol regulatory element binding proteins) are synthesized to increase MK activity, and then cholesterol appears to be a main "sensor" of the pathway (79).

The role of this metabolic defect on inflammation is unclear but it has been demonstrated that the shortage of isoprenoid end products increases IL-1 β secretion in MK-deficient PBMC (peripheral blood mononuclear cells) (80). Moreover, stimulated lymphocytes of MKD patients show decreased apoptosis. The therapeutic options for MKD are still limited and most experience relies on case reports. Some patients may respond to high-dosed steroids when given at crises onset. More recently clear success has been obtained in patients with either HIDS or MKD with anakinra, the IL-1-Ra, which targets IL-1. The use of statins, inhibiting HMGR, has been shown to aggravate patients with MA, by limiting the flux in the pathway. Other products inhibiting the isoprenoids like ZAA (zaragotic acid A, a squalene inhibitor) or biphosphonates could activate the feed back and induce the transcription of MK. Severe cases of MA could be cured by allogenic bone-marrow transplantation (81). In all cases, a low-cholesterol diet is recommended.

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