

Meeting Report

An update on autoinflammatory diseases. New concepts for new and old diseases

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Abstract

The discovery of MEFV as the susceptibility gene for autosomal recessive Familial Mediterranean Fever (FMF) in 1997 represents the beginning of the new era of the monogenic autoinflammatory diseases. During the last decade, the increasing knowledge on the pathogenic mechanisms related to a number of diseases associated to mutations of genes associated to autoinflammatory diseases had a terrific impact on the understanding of pivotal mechanisms regulating the inflammatory response and therefore represents one of the major advance in the field of inflammation.

The International Congress on Familial Mediterranean Fever and Systemic Autoinflammatory Diseases brings together the experts in the field every two and a half years and represents a unique opportunity for an update on the recent progress in this growing field. The fifth edition of the congress was held in Rome (Italy, 4-8 April 2008). Most of the contributions to this meeting have been published during the course of the present year. Thus, the aim of the present article is to report the main highlights from the above-mentioned meeting and to give a general update of the more recent advances in this field.

Introduction

Autoinflammatory diseases are a group of different conditions secondary to mutations of gene coding for proteins that play a pivotal role in the regulation of the inflammatory response. The clinical spectrum of these disorders is extremely variable (Table I) and will likely enlarge in the near future (1, 2). During the last decade, the increasing knowledge on the pathogenic mechanisms related to mutations of genes associated to autoinflammatory diseases had a terrific impact on the understanding of pivotal

mechanisms regulating the inflammatory response.

The Fifth International Congress on Familial Mediterranean Fever and Systemic Autoinflammatory (Rome, 4-8 April 2008) was a precious opportunity to update of the current knowledge in the field.

There were two introductory lectures. In the first, Daniel Kastner (Bethesda, USA) gave an overview of the expanding field of the autoinflammatory diseases (new levels of complexity for Mendelian disorders, new insights on the molecular pathophysiology of autoinflammation, new animal models, development of small molecules for therapeutic purposes). In the second, J. van der Meer (Nijmegen, The Netherlands) updated the differential diagnosis of fever of unknown origin including new diagnostic tools (*i.e.* FDG PET scanning, 16S rRNA amplification).

Pathways of the innate immunity

The first part of the meeting was devoted to an update on the major advancements in the understanding of the different pathways of activation of the innate immunity and its effector functions.

The alteration of the mechanisms regulating the recognition and response to exogenous stimuli such as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) may cause an inappropriate activation of cells of the innate immunity and represents the primary event leading to a severe local and/or systemic inflammation.

A review on the role of Toll-like receptors (TLRs) in the recognition of conserved non-self molecular signatures, carried by microorganisms, was addressed by D. Golenbock (Worcester, USA). Toll-like receptor 4 is triggered by most lipopolysaccharides (LPS) derived from Gram-negative bacteria.

Competing interests: none declared.

Table I. The autoinflammatory diseases.

	Diseases	Gene Chromosome	Protein	Transmission	Clinical features
Periodic/recurrent Fevers	Familial Mediterranean fever	<i>MEVF</i> 16p13.3	Pyrin	AR	Short duration of fever episodes: 24-48 hours. Abdominal and chest pain. Erysipelas-like erythema. High incidence of renal amyloidosis in untreated patients. Good response to colchicine. Possible response to IL-1 blockade.
	Mevalonate kinase deficiency	<i>MVK</i> 12q24	Mevalonate kinase	AR	Early onset (usually <12 months). Mean duration of fever episodes: 4-5 days. Poor conditions during fever episodes. Abdominal pain, vomiting and diarrhoea. Splenomegaly. Good response to steroids. High rate of self-resolution during adulthood. Amyloidosis is rare.
	TNF receptor associated periodic syndrome	<i>TNFRSF1A</i> 12p13	p55 TNF receptor	AD	Prolonged fever episodes: 1-3 weeks. Periorbital edema, monocytic fasciitis. Incidence of renal amyloidosis. Response to TNF- and IL-1 blockade
NALPs-related disorders	FCAS, MWS, CINCA	<i>CIAS 1/NALP3</i> 1q44	Cryopyrin	AD	FCAS: rash, fever and arthralgia after cold exposure. MWS: recurrent or sub-chronic urticaria-like lesions. sensorineural hearing loss, amyloidosis CINCA: as above + mental retardation, chronic aseptic meningitis and bone deformities. Good response to IL-1 blockade.
	NALP12-associated Periodic Fever	<i>NALP12</i>	NALP12	AD	Periodic fever after cold exposure, hearing loss.
Granulomatous disorders	Blau's syndrome	<i>CARD15/NOD2</i> 16q12	CARD15	AD	Early onset (<5 years). Polyarticular granulomatous arthritis, uveitis, skin rash Good response to anti-TNF monoclonal antibodies
Pyogenic disorders	PAPA syndrome	<i>PSTPIP1</i> 15q24-q25.1	PSTPIP1	AD	Pyogenic sterile arthritis, pyogenic gangrenosum, Cystic acne. Good response to IL-1 blockade.
	Majeed's syndrome CRMO (murine)	<i>LPIN2</i> 18p <i>PSTPIP2</i> 18p	LPIN2 PSTPIP2	AR AR	Multifocal osteomyelitis, congenital dyserythropoietic anemia, inflammatory dermatosis

FCAS: Familial Cold Autoinflammatory Syndrome; MWS: Muckle-Wells Syndrome; CINCA: Chronic Infantile Neurological Cutaneous and Articular Syndrome; PAPA: Pyogenic Sterile Arthritis, Pyoderma Gangrenosum and Acne (PAPA) syndrome; CRMO: chronic recurrent multifocal osteomyelitis; AR: autosomal recessive; AD: autosomal dominant.

*Identification of the gene defect.

MD2 is a small, secreted glycoprotein that acts as an extracellular adaptor binding to both the hydrophobic portion of LPS and to the extracellular domain of TLR4. Recently, soluble MD-2 has been showed to acts as an opsonin. In fact it is able to bind tightly to the surface of live gram-negative bacteria, thus enhancing cellular activation, bacterial internalization, and intracellular killing, all in a TLR4-dependent manner (3).

Trauma and other cellular stresses are recognized at the cell level via receptor-mediated detection of intracellular proteins released by the injured cells. The term “alarmin” has been proposed to categorize such endogenous mole-

cules that signal tissue and cell damage. Endogenous alarmins and exogenous PAMPs convey a similar message and elicit similar responses; they can be considered subgroups of a larger set, the damage-associated molecular patterns (DAMPs) (4). M Bianchi (Milan, Italy) discussed the role of High Mobility Group Box 1 (HMGB1) in many pathologic conditions (sepsis, trauma, rheumatoid arthritis, tumour metastasis). HMGB1 acts as an alarmin; it is released by necrotic cells and actively secreted by inflammatory cells via a non-classical pathway, and exerts a number of pro-inflammatory activities, such as attracting inflammatory cells, recruiting stem cells and promoting

their proliferation, activating dendritic cells and promoting their functional maturation (5).

One of the most important downstream effects of the dysregulation of innate immunity observed in the autoinflammatory diseases is an un-appropriate activation of caspase 1 leading to an over-secretion of interleukin-1 (IL-1). The inflammasome play a crucial role in IL-1 β processing through the activation of caspase-1, which in turn converts pro-IL-1 β to the mature, active 17 kDa form. A. Rubartelli (Genoa, Italy) reviewed the pattern of production and secretion of IL-1 β . Unlike most cytokines, IL-1 β lacks a secretory signal peptide and is externalized by

monocytic cells through a non classical pathway, arranged in two steps (6, 7). First, Toll-like receptor ligands, such as LPS, induce gene expression and synthesis of the inactive IL-1 β precursor (pro-IL-1 β). A second stimulus, such as exogenous ATP, strongly enhances proteolytic maturation and secretion of IL-1 β (8). ATP is released by circulating monocytes following activation of various pathogen-sensing receptors and autocrinally induces IL-1 and IL-18 secretion by monocytes themselves (9). ATP-triggered IL-1 β secretion is mediated by P2X7 receptors expressed on the surface of monocytes (10). Monocytes carrying mutated NALP-3 over-secrete IL-1 in response to signal 1 (LPS) but do not respond to signal 2 (ATP), indicating that in NALP-3 mutated monocytes a single stimulus (danger signals released by injured cells or bacterial products) even if in low amounts unable to trigger IL-1 secretion in healthy individuals, is sufficient to drive a dramatic inflammatory cascade (11).

F. Martinon (Lausanne, CH and Boston, USA) highlighted the recent discovery that monosodium urate monohydrate (MSU) crystals may behave like DAMPs activating the NALP3 inflammasome represents. Indeed in monocytic cell line or peripheral blood monocytes from normal volunteers cleave proIL-1 β and release the active 17-kd molecule after exposure to MSU crystals. Peritoneal macrophages from mice genetically deficient in the components of the NALP3 inflammasome failed to process proIL-1 β after MSU stimulation in comparison to wild-type mice, showing the pivotal role of the inflammasome in this process (12). Notably preliminary experiences have shown the efficacy of IL-1 blockers in acute and chronic gout, (13, 14).

Further possible exogenous triggers for the inflammasome has been reviewed by R.A. Flavell (New Haven, USA). NALP3 has been identified as an important innate immune receptor involved in the recognition of silica and asbestos by macrophages. Stimulation of macrophages with either silica or asbestos resulted in the robust secretion of IL-1 β in a manner dependent on the NALP3 inflammasome. Chronic

fibrosis seen in a murine model of silicosis is dependent upon the presence of inflammasome-components, ASC and NALP3. These findings suggest that silica-induced production of IL-1 through the inflammasome plays an important role in the initial inflammatory responses that lead to silicosis (15). The same effect on inflammasome has also been shown for aluminium adjuvants, thus revealing the pivotal role of the inflammasome pathway in directing the humoral adaptive response (16).

The large family of IL-1 cytokines has been reviewed by C. Dinarello (Denver, USA). These proteins share a common β -barrel pattern consisting of 12 β -strands and significant amino acid homology with the IL-1 receptor antagonist (IL-1Ra), IL-1 β , and IL-18. The new members of the IL-1 family are derived from a common ancestor, as are IL-1 and IL-18. On the basis of their structure, these IL-1 family members potentially can act as agonistic or antagonistic ligands for members of the IL-1 receptors family; however, their biological function is presently unknown. The functional properties of a less known IL-1 like cytokine with antagonist activity, IL-1F7, has been recently clarified (17). IL-1F7 is constitutively expressed in most human tissues and circulating monocytes and has five different splice variants (IL-1F7a-e). IL-1F7b shares significant sequence homology with IL-18. It contains an instability sequence, which limits its induction in macrophages in normal condition. When induced by TLR ligands, exerts a profound suppressive effect on LPS-induced secretion of TNF- α , IL-1 α , IL-6 and MIP-2 but not MIP-1 α . IL-1F7 binds with a low affinity to the IL-18R alpha chain and also to the IL-18 binding protein, thus inhibiting IL-18 activity (17).

The functional interaction of some pivotal components of the innate immunity (*i.e.* proteins of the NALP family, NOD2/CARD15) with the adaptive immune response and the development and maintenance of autoimmunity was presented by M. McDermott (Leeds, UK). Interestingly, SNPs of the *NALP1* gene are associated with several vitiligo-associated autoimmune disorders

(18), implicating innate immunity in the pathogenesis of these disorders. On the other hand, NALP3 (cryopyrin), ASC, and pyrin mRNA expression are significantly increased in rheumatoid arthritis (19). According to ongoing studies baseline mRNA synovial tissue NALP3 expression may act as a potential predictor of response to infliximab in RA patients.

NALPs-related diseases

NALP proteins represent a large family of proteins characterized by the same structure and exerting a number of different cellular activities (20). Muckle-Wells Syndrome (MWS, MIM 191900), Familial Cold Autoinflammatory Syndrome (FCAS, MIM 120100) and Chronic Infantile Neurological Cutaneous and Articular Syndrome (CINCA, MIM 607115) represent autosomal dominant disorders characterized by different mutations in the gene of NALP-3 (or *CIAS1*, or *PYPAFI*), encoding a protein called cryopyrin (21). R. Goldbach-Mansky (Bethesda, USA) and Hal Hoffman (S. Diego, USA) gave an exhaustive overview on the clinical picture of NALP3-related diseases, with a very interesting update on the response to different IL-1 blockers. In particular, Goldbach-Mansky reported on the long term efficacy of anakinra treatment in CINCA /NOMID patients showing the follow-up of the data recently reported in a multicenter north-American study (22). To date 20 NOMID patients have been treated with IL-1 receptor antagonist (anakinra) for a three-year period. The drug is reported to be persistently effective in controlling systemic inflammation. However, an escalation in the dosage up to 4.5 mg/kg/day was needed in 17 out of 20 patients. An increased intracranial pressure was still present in 60% of patients. Notably, CSN inflammation and increased intracranial pressures were often (50% of cases) not associated with systemic inflammation. A significant improvement of motor and functional scores was also observed. At 36 months, improvement of hearing loss was obtained in 5 out 18 (28%) patients, whereas 50% of them were unchanged (4 patients) or worsened (5 patients). A general improve-

ment in visual impairment was also observed. Despite anakinra treatment a progression of bony lesions growth was observed. Thus, despite the significant improvement in the quality of life of CINCA/NOMID patients, further attempts has to be made to increase the long term prognosis of these patients.

H. Hoffman reported on the efficacy and safety of a new IL-1 blocker, rilonacept in patients with FCAS. Rilonacept is a dimeric fusion protein (251 kDa) that is a specific blocker of the two components required for IL-1 signaling (IL-1 receptor sub-type 1 and IL-1 receptor accessory protein). The drug has a half-life of 8.6 days. Forty-seven patients with FCAS and Muckle-Wells were involved in two sequential studies. The use of rilonacept (160 mg/week) showed a dramatic amelioration of the clinical score and of laboratory parameters (23). Similar results were obtained in a parallel study from the NIH's group (24).

I. Aksentijevich (Bethesda, USA) presented a microarray-based gene expression study of systemic inflammation in patients with cryopyrin-associated periodic syndromes (CAPS); goal of this study was to develop a model-based classifier able to accurately predict whether patients are likely to have CAPS based on their gene expression profile. A significant number of differentially expressed genes (DEG) were found many of which involved in the regulation of NF- κ B pathway. The model based gene expression signature was very accurate in predicting both CAPS and non-CAPS patients (25). S. Brydges (Bethesda, USA) reported on the initial characterization of a mouse model of cryopyrinopathy. Heterozygous mice for A352V mutation of *NALP-3* gene (associated in humans with Muckle-Wells phenotype) show a profound growth delay, with significant elevation of circulating neutrophils count and neutrophil tissue infiltration with respect to wild-type mice. Mice showed a very short survival, with a mean of 9 days (26). Consistent with data from humans (11), peritoneal macrophages showed a hyper-responsiveness to LPS that was independent from ATP stimulation.

Interestingly, mutations of another member of the NALP-family, have been recently associated with recurrent hydatidiform mole and reproductive wastage in humans (27). R. Slim (Montreal, Canada) reported on the possible link between this component of NALP family and the reproductive alteration. Notably, in animals, intrafollicular injection of IL-1 β induces ovulation, but embryos arrest during early cleavage. Moreover, IL-1 β is involved in embryo-maternal signaling, trophoblast invasion, and proliferation (28).

A special oral communication was dedicated to the novel finding of NALP12 as a new gene associated to an autoinflammatory disease. I. Jeru (Paris, France) reported on two families with a clinical phenotype similar to FCAS (induction by cold) and Muckle-Wells syndrome (sensorineural hearing loss) and presenting a non-sense and one splice heterozygous mutations of NALP12, respectively (29). NALP12 (or Monarch-1 or PYAPF7) plays an important role as an inhibitor of the inflammatory response (NF- κ B signaling) (30). The functional consequences of the identified NALP12 mutations (decreased inhibitory activity on NF- κ B activation compared to wild-type protein) were also shown (29).

From MEFV to familial Mediterranean fever

Familial Mediterranean fever (FMF, MIM 249100) is the prototype of the hereditary autoinflammatory disorders. It is characterized by recurrent attacks of fever and/or serosites and affects mainly people living around the Mediterranean basin (extremely high carrier frequency), even if cases from all over the world were recently reported. The discovery of the causative gene (MEFV) allowed progresses in understanding either the molecular basis of FMF and, in general, some pivotal mechanisms regulating the inflammatory cascade.

J. Chae (Bethesda, USA) gave an overview on the available mouse model for FMF. Notably the knock-in mice with FMF-associated mutants presents similar inflammatory phenotypes to FMF, with differences in the severity of inflammation according to the

genotype (WT-B30.2 >V726A >M680I >M694V)(31). Conversely, the knock-out mice has heightened sensitivity to endotoxin (32). A number of evidences with these models indicate an important role for pyrin in the regulation of caspase-1 and IL-1 β activation (31, 32). In a recent study, the same group provides evidences on the capacity of caspase 1 to directly cleave pyrin producing a 330-residue N-terminal fragment that enhances ASC-independent NF- κ B activation (31).

I. Touitou (Montpellier, France) discussed the possible pitfalls in the interpretation of molecular analysis of MEFV. She also suggested how to report about polymorphisms and very rare sequence variants and to define a test as "negative" or "excluding the diagnosis" only considering the actual exhaustiveness of the screening strategy. Moreover, she stressed the concept that, in the presence of a single mutation, the disease cannot be excluded, such as, in case of two mutations, a recessive disease should be confirmed testing both parents. The preliminary results on a project aimed to improve the quality of gene testing in periodic hereditary fevers have been also reported.

S. Ozen (Ankara, Turkey) discussed the issue of *MEFV* heterozygosity, which accounts for 20-26% of the patients with a clinical diagnosis of FMF. Different mechanisms could be proposed: i) other unidentified mutations (*i.e.* in the promoter region?), ii) other causative gene, iii) psychological influences in siblings of an affected child, iv) digenic inheritance among autoinflammatory disorders, v) polymorphisms in other relevant genes (SAA1, MICA, TLR2), v) environmental factors (as suggested by the lack of amyloidosis in Armenians living in US or different disease severity in patients of the same ethnicity living in different Countries) (33, 34). From the practical point of view, heterozygotes MEFV patients positive for FMF criteria and/or displaying elevated serum A amyloid should be treated with colchicine, when other inflammatory or autoinflammatory disorders have been excluded.

R. Manna (Rome, Italy) reviewed the current evidence that MEFV mutations

can protect against asthma, whereas they may worsen the severity of rheumatoid arthritis (RA), modify inflammatory bowel diseases (IBD) phenotype, facilitate a chronic protracted myalgia syndrome and a possible progression towards a cryptogenetic cirrhosis.

A. Livneh (Tel-Aviv, Israel) reviewed the traditional treatment of FMF with colchicine, while H. Ozdogan (Istanbul, Turkey) discussed possible alternative drugs (interferon, thalidomide, anti-TNF and anti-IL1) in colchicine-resistant patients and their possible indication in some particular FMF-associated manifestations such as vasculitis, chronic arthritis and amyloidosis. Unfortunately, few randomized controlled trials are available, to date. A trial with a new IL-1 blocker (Rilonacept) in FMF is ongoing.

S. Stojanov (Bethesda, USA) showed that peritoneal cells of pyrin-null mice display a reduced expression of CD11c, a marker for dendritic cells, thus providing evidences of an aberrant development of dendritic cells in these mice (35). G. Wood (Bethesda, USA) studying human monocytic cell line demonstrated that knockdown of pyrin causes down regulation of TLR4 activation an effect that appears to be mediated by IRF2 (36).

T. Kallinich (Berlin, Germany) presented the results of a study on S100A12 serum level. S100 is a protein expressed mainly by granulocytes, which stimulate up-regulation of adhesion molecules and secretion of proinflammatory cytokines. The study shows that S100A12 may act as a new sensitive marker for disease monitoring in FMF. In fact, between fever attacks episodes S100A12 seems to be more sensitive than the classical markers in the detection of subclinical inflammation (37).

M. La Regina (Rome, Italy) described a multi-center survey on colchicine therapy in more than 4000 patients from 8 different countries. A diffuse heterogeneity concerning dosages, administration and schedules (efficacy and toxicity monitoring, definition of intolerance and resistance, policy related to pregnancy, proteinuric patients, etc.) was observed. The elaboration of guidelines is still needed (38).

F. Yalcinkaya (Ankara, Turkey) suggested a new set of criteria for FMF diagnosis in children, based on a cohort of genetically confirmed patients. These criteria showed an high sensitivity and specificity in Turkish population and should be validated in other ethnic groups (39).

E. Verrecchia (Rome) demonstrated that small intestinal bacterial overgrowth (SIBO) can reduce colchicine responsiveness, and that bowel decontamination improves response to colchicine (40).

From mevalonate kinase deficiency to periodic fever

The periodic fever associated with mevalonate kinase deficiency (MKD) was originally identified in 1984 in six patients of Dutch ancestry with a long history of recurrent attacks of fever of unknown cause and a high serum IgD level (41). For this reason this disorder has also been named Hyper IgD syndrome or Dutch fever. High IgD plasma levels have been used as a diagnostic hallmark until when mutations in the mevalonate kinase (MVK) gene, encoded on chromosome 12q24, were identified as the cause of the disease (42).

J. Drenth (Nijmegen, the Netherlands) gave an exhaustive update on the clinical aspects of this disease. At present, almost 300 MKD patients have been reported worldwide. Most of the cases come from Western Europe, even if sporadic cases has been reported also in Asia and in the ethnic groups presenting an high prevalence of FMF (Turks, Arabs). In the Dutch population the frequency of carriers of MVK mutation is relatively high, 1:65. However the incidence of the disease is much lower than expected (0.02-0.002%) thus suggesting a low penetrance of some mutations. High IgD plasma levels (>100 UI/ml) have been used as a diagnostic hallmark of the disease. However, the sensitivity and specificity of these findings is debated. In a recent series of 50 patients, the sensitivity of a high IgD value for the diagnosis of HIDS was 0.79 but the specificity of this test was much lower (0.27), since 19 out 38 healthy individuals displayed elevated IgD levels (43). Anakinra has been

shown to be markedly effective in 5 different case reports. So far, a total of 9 patients were treated with anti-TNF treatment (etanercept) with a variable range of response (4 good, 4 partial, 1 absent). In comparison to other periodic fevers, amyloidosis has been confirmed to rarely affect patients with MKD. So far, only 4 MKD patients with this complication have been reported.

J. Frenkel (Utrecht, the Netherlands) gave an overview on the possible pathogenic mechanisms by which the reduced activity of MKV leads to inflammatory flares. MKV catalyzes the ATP-dependent phosphorylation of mevalonate to 5-phosphomevalonate, and is first enzyme that follows the highly regulated enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase in the isoprenoid biosynthesis. Two main hypotheses have been formulated. The first is that inflammation is related to elevated mevalonic acid levels. This hypothesis comes from the clinical observation of a reduction of the severity and frequency of fever episodes in 6 adult MKD patients treated with simvastatin. Like other statins, simvastatin is a competitive inhibitor of HMG-CoA reductase, thus this treatment would lower mevalonate levels with a consequent reduction of inflammation. In contrast with this hypothesis, mevalonate has been reported to decrease and not to increase, the *in vitro* IL-1 β secretion from monocyte. A second hypothesis is that a shortage of isoprenoid end products, rather than an excess of mevalonate, contributes to the inflammation in MKD. In fact, the shortage of the nonsterol isoprenoid end products, mainly the geranylgeranyl groups, leads to an increased activation of caspase 1 in circulating monocytes with a consequent hypersecretion of the 17 Kd active form of IL-1 β . LM Kuijk (Utrecht, the Netherlands) reported on the possible molecular mechanisms regulating simvastatin-induced activation of caspase-1 and subsequent increased IL-1 β release. The study shows that simvastatin treatment results in caspase-1 activation through a Rac1/PI3K/PKB dependent pathway and that inhibition of Rac1 can decrease IL-1 β release by MKD PBMC (44).

J. Ch. van der Hilst (Nijmegen, the Netherlands) reported on the follow-up, clinical features and quality of life of 103 MKD patients included in the International registry held by the Nijmegen's group. MKD is generally thought as a self-limiting and benign disease with a long-term good prognosis. However, according to this experience, 23.9% and 17.8% of patients still present more than 12 attacks per years in the second decade of life and after 20 year of age respectively. Moreover, MKD impaired several aspects of the quality of life. Social functioning, general health perception, vitality, autonomy and social development were significantly lower than controls (45). One of the most relevant issues related to periodic fever is associated with the identification of those patients at higher risk to carry relevant mutations of genes associated with periodic fever. S. Federici (Genoa, Italy) presented the results of a recent study conducted on 228 consecutive children with a clinical history of periodic fever that were screened for mutations of *MVK*, *TNFRSF1A* and *MEFV* genes. The statistical analysis allowed the identification of some clinical variables (positive family history, early age at onset, presence of abdominal and chest pain, diarrhea, absence of aphthosis) that are highly associated with the probability to carry relevant mutations in already known genes. The study was also oriented to the identification of the clinical clues able to indicate the most reasonable order of the genes to be screened in patients with a high risk to be positive at the genetic test. The duration of fever from 3 to 6 days, the presence of splenomegaly and vomiting was highly evocative for MKD. Based on the previous results a flow-chart has been proposed as a guideline for genetic testing in children with recurrent fever of unknown origin ((46) see also www.printo.it/periodicfever).

New insights for TRAPS

The TNF-receptor associated autoinflammatory syndrome (TRAPS) was formerly known as familial Hibernian fever, since it was initially described in a kindred of Irish-Scottish descents in

1982 (47). It is a dominantly inherited disorder, caused by mutations in the p55 TNF Receptor (or TNFR1), encoded by the TNF Super Family Receptor 1A gene (*TNFRSF1A*) (48).

Different pathogenic mechanisms have been proposed for TRAPS. It was originally hypothesized that TRAPS develops as a consequence of impaired down-regulation of membrane TNFR1 and diminished shedding of this soluble receptor (48). However, some *TNFRSF1A* mutations are not associated with defective shedding of the p55 TNFR1 (49), suggesting that additional mechanisms are involved in the pathogenesis of the disease. The functional impact of the structural mutations of TNFR1 has also been actively investigated using transiently transfected cells as model system. Impaired trafficking of the defective TNFR1 to the cell membrane (50-52) with accumulation of the protein in the endoplasmic reticulum (ER) (53) has been demonstrated. The actual relevance of TNFR1 defective function, as detected in transfected cells, on the homeostasis of primary cells from TRAPS patient is still matter of debate and investigation. During the meeting a number studies, performed with different experimental approaches, were discussed.

A. Simon (Nijmegen, Netherland & Bethesda, USA) focused on the comprehension of the pro-inflammatory pathogenic consequences of the "entrapment" of mutated TNFR1 in the ER. The study was performed on different models of endogenous mutated TNFR1 (PBMC from TRAPS patients, knockin mice and mouse embryonic fibroblasts (MEF)). The Western-blot analysis confirmed the presence of an intracellular accumulation of TNFR1 in patients carrying structural *TNFRSF1A* mutations. According to this study, the accumulation of TNFR1 leads to a pro-inflammatory increased signaling that involves the MAPK activity (pJNK), but not the NF- κ B pathway. Simon *et al.* suggest that the abnormal activation of pJNK is related to the increased production of reactive oxygen species (ROS) that was observed in TRAPS MEFs and human PBMCs in the presence of LPS.

I. Todd (Nottingham, UK) reported on

the recent studies showing the structural and antigenic changes caused by mutations of the first CDR of the *TNFRSF1A* gene. The use of two distinctive antibodies with different antigenicity (one specific for both WT and mutated receptor and the second for WT form only) showed that, at least for some mutations (*i.e.* C33Y) most of surface and secreted TNFR1 is represented by the WT and not by the mutated receptor (54).

M. Gattorno (Genoa, Italy) reported on the unpublished observations of his group based on the study on a large number of Italian TRAPS patients. This group analyzed the capacity of internalization of TNFR1 after TNF- α stimulation in TRAPS patients and healthy controls. Notably, the compartmentalization and internalization of TNFR1 is thought to play a crucial role in the activation of the pro-apoptotic pathway. TRAPS patients showed an impaired internalization of the TNFR1 receptor that could be one of mechanisms associated to the defect of TNF-induced apoptosis observed in these patients (55). The recent favorable experience of this group on the long term efficacy of Anakinra in 5 TRAPS patients was also reported (56).

B. Nedjai *et al.* (London, UK) reported on the recent observation coming from patients with the fully penetrant C73R mutation who had marked activation of the pro-inflammatory p65 subunit of NF- κ B. In contrast with other mutations, cells from patients with the C73R mutation have no TNFR1 shedding defect; there was, nonetheless, an unusually high concentration of functional TNFR1 at the plasma membrane (57).

The analysis of gene-expression signatures in peripheral blood mononuclear cells of 37 patients with TRAPS and 38 age- and gender-matched controls was reported by J. Ryan (Bethesda, USA). In their study, the changes in gene expression levels in TRAPS patients were relatively subtle despite a substantial number of samples. The pathway analysis identified TNF and NF- κ B, as major pathways involved in the pathogenesis of disease. According to the Authors, future modelling with microarrays from patients with

other autoinflammatory diseases will identify the genes that are specific for TRAPS (58).

Other monogenic autoinflammatory diseases

C. Wouters (Leuven, Belgium) gave an overview of the clinical features of Blau's syndrome, or early onset sarcoidosis, an autosomal-dominant inflammatory disease associated to mutation of the NACHT domain of *NOD2/CARD15* gene (59) and characterized by a noncaseating granulomatous inflammation affecting the joint, the skin, and the uveal tract (60). An update of the International Registry on Pediatric Granulomatous arthritis was given (61). So far 118 individuals (47 pedigrees) have been collected. 26 pedigrees (37 individuals) displayed the classical phenotype associated with *NOD2/CARD15* mutations (prevalently R334Q and R334W substitutions). Together with the classical triad, other rarer manifestations (hepatic granulomata, lymphadenopathy, parotid gland, erysipelas-like rash, glomerulonephritis and interstitial nephritis, splenomegaly and arterial hypertension) have been observed. Interestingly, a subgroup of five patients, negative for mutations of the *NOD2/CARD15* gene were characterized by an early-onset and a painful panniculitis; they may represent a distinct clinicopathologic entity or may be part of the spectrum of pediatric granulomatous inflammatory diseases (62).

The Pyogenic Sterile Arthritis, Pyoderma Gangrenosum and Acne (PAPA) syndrome, is an inflammatory disease caused by mutations of the gene coding for the Proline Serine Threonine Phosphatase Interacting Protein 1 (PSTPIP1) (63). D. Kastner (Bethesda, USA) overviewed the clinical manifestations characteristic of this extremely rare disease, consisting of episodic pyogenic sterile arthritis, pyoderma gangrenosum and cystic acne (64). The good response to both anti-IL-1 and anti-TNF treatments was also discussed on the bases of the few available case reports. Studies from the Bethesda' groups elegantly showed that PSTPIP1 functionally interacts with Pyrin (FMF). The binding is regulated by the phosphor-

ylation status of PSTPIP and PAPA-associated mutations result in increased phosphorylation and thus increased pyrin binding (65). An alternative hypothesis has been recently proposed by Yu and co-workers. According to their studies, ligation by PSTPIP1 activates pyrin by unmasking its PYD, thereby allowing it to interact with ASC and facilitate ASC oligomerization into an active ASC pyroptosome. PAPA-associated PSTPIP1 mutants were found to be more effective than WT PSTPIP1 in inducing pyrin activation (66) that, according to these last Authors, would be associated with a pro-inflammatory, rather than a modulatory effect on Caspase 1 activity (67).

The presence of sterile pyogenic abscesses with a prevalent bone localization is also the hallmark of another very rare autosomal recessive disease observed in families of Arabian origin, the Majeed's Syndrome. H. El Shanti (Iowa City, USA) reviewed the clinical features of this conditions characterized by chronic recurrent multifocal osteomyelitis (CRMO) associated with congenital dyserythropoietic anemia and inflammatory dermatosis (68); the disease is due to mutation of the *LPIN2* gene (69). The existence of a monogenic disease associated with CRMO has raised the hypothesis that also sporadic CRMO could be included in the group of autoinflammatory diseases. This hypothesis have been further supported by the recent identification on a chromosome 18 of a missense mutation in the *pstip2* gene in spontaneous murine model of chronic multifocal osteomyelitis (70).

The expanding spectrum of the autoinflammatory diseases

A relevant part of the meeting was devoted to an update on a number of clinical conditions so far considered as multifactorial, that due to their clinical features and response to treatment are supposed to represent autoinflammatory diseases.

M. Hofer (Lausanne, Switzerland) gave an update on an international Registry that has collected information on 214 patients fulfilling the PFAPA (Periodic fever, aphthous stomatitis, pharyngitis

and adenitis) criteria. The analysis of this cohort of patients allowed some interesting considerations on the actual utility of these clinical criteria (71). A comparison between patients with a complete (presence of all three clinical manifestations) and incomplete phenotype was also reported. Patients with a complete phenotype displayed a higher incidence of skin rash, diarrhea and myalgia. Another important criteria is related to the exclusion of other periodic fevers associated to mutations of already known genes (*MEFV*, *MVK*, *TNFRSF1A*). In this line an increased utilization of molecular testing according to specific clinical manifestations has been recommended. Finally, the study revealed the overall good response to tonsillectomy in the 35 patients that underwent to this procedure (27 resolution of fever attacks, 1 improvement, 6 stable, 1 worsened).

A number of clinical, immunological and demographic issues reinforce the hypothesis that also Behçet's disease (BD) could be considered an autoinflammatory disease. The pros and cons of this hypothesis have been clearly analyzed by A. Gül (Istanbul, Turkey). BD has a geographic distribution similar to FMF and an increased frequency of *MEFV* mutations has been described in BD patients. Moreover, a number of clinical features of BD display a clear overlap with those of hereditary autoinflammatory disorders; they include oral aphthous ulcers (MKD, TRAPS, MWS), acne-like lesions (PAPA syndrome), uveitis (CINCA/NOMID, Blau), meningoencephalitis (FMF, NOMID, MWS), orchy-epididymitis (FMF), pathergy reaction (PAPA), arthritis and amyloidosis (all diseases). On the other hand, BD is a complex genetic disorder strongly associated with HLA Class I (HLA-B51) and T cell-specific treatments (*i.e.* cyclosporine A) may be effective.

I. Konè-Paut (Kremlin-Bicêtre, France) presented the clinical characteristics of pediatric BD and the possible pitfalls in the diagnostic approach to the disease in childhood, taking into account the general onset during the second decade and the high number of incomplete cases according to the current adult diagnostic criteria. An international multi-

center prospective study on this specific aspect (Ped-BD) is now in progress.

In the context of the large clinical spectrum characterizing the different forms of juvenile idiopathic arthritis (JIA), the systemic onset subtype shares a number of clinical (absence of gender prevalence and HLA association, presence of fever and systemic manifestations) and pathogenic features with the autoinflammatory disease (72). A. Martini (Genoa, Italy) reviewed the clinical picture of this condition, the experimental evidences showing the pivotal role of IL-1 and of the downstream IL-6 in the pathogenesis of the disease (73, 74) as well as the promising results of a recent randomized placebo-control study with anti-IL-6R mAb (Tocilizumab) (75). Moreover, according to a recent study from the group of Genoa, the dramatic response to IL-1 blockade is able to identify a subset of systemic onset JIA patients characterized by a milder joint involvement (76).

The more recent advances on Schnitzler syndrome has been outlined by Dr. A. Simon (Nijmegen, The Netherlands). This syndrome is characterized by the late onset (median age 50 years) of a chronic urticarial rash and a monoclonal IgM or IgG gammopathy associated with recurrent fever, arthralgia, arthritis, bone pain and bone abnormalities. Even if the late disease onset and the absence of familiar cases are not associable to the monogenic autoinflammatory conditions, the overall clinical manifestations and response to treatment suggests including this condition in the group of autoinflammatory diseases. So far, data on 94 patients have been collected (77). After a mean follow-up of 10 years, almost 15% of patients developed Waldenström's macroglobulinemia. Development of AA amyloidosis has also been observed. Treatment with anakinra was dramatically effective in the control of the inflammatory manifestations, whereas the disappearance of the gammopathy and a complete long lasting remission was anecdotally reported after rituximab use (78).

A. Brucato (Bergamo, Italy) reported on another clinical entity, recurrent pericarditis characterized by periodic acute attacks of isolated acute pericar-

ditis associated with elevation of acute phase reactants and circulating neutrophils. Familial occurrence has been reported in up to 10% of patients (79). Colchicine represents the first-choice therapy for recurrent pericarditis resistant to conventional anti-inflammatory treatment. In a prospective randomized open label study, 84 patients with recurrent pericarditis the conventional treatment (ASA: 800 mg orally every 6 or 8 hours or oral prednisone, 1mg/kg/die) was compared to the conventional treatment plus colchicine. The recurrences rate at 18 months was 51% in the first group and 24% in the colchicine-treated group. Notably, previous corticosteroid use was an independent risk factor for further recurrences (80). Brucato also reported on a recent study in which "low doses" (0.2-0.5mg/kg/day) of prednisone were compared with the standard regimen of 1 mg/kg/day in 100 patients. A significant reduction of side effects, recurrences and hospitalization was observed in the arm treated with low-doses of steroid. The efficacy of anakinra in steroid-dependent and colchicine-resistant patients with recurrent pericarditis has been recently reported (81), suggesting that at least some form of recurrent pericarditis may represent an autoinflammatory disease.

Recurrent aseptic abscesses (H. André, Clermont-Ferrand, France) represent another unusual clinical entity possibly related to the large spectrum of autoinflammatory conditions. Patients display periodic fever episodes associated with abdominal pain. Abscesses are usually observed in the spleen, with a possible co-localization in liver, abdominal lymph nodes, pancreas and lung. This condition may be associated with a number of inflammatory diseases (inflammatory bowel diseases, relapsing polychondritis, spondyloarthritis, monoclonal gammopathy of undetermined significance), but an idiopathic form can be observed in 10% of patients (82). Histology shows sterile round lesions with a necrotizing core containing neutrophils surrounded by palisading histiocytes and giant cells. Cultures from blood and the lesions are constantly negative. Abscesses do not respond to antibiotic treatment but

shows a dramatic response to steroids. Relapses has been observed in almost 60% of the patients (82).

Evidences for new autoinflammatory diseases

Together with the above mentioned identification of NALP12 as a new gene possibly associated to an autoinflammatory condition (29), other reports suggested the identification of new types of autoinflammatory diseases.

A. Rösen-Wolff (Dresden, Germany) reported on the association of structurally altering mutations of caspase-1 with periodic fever (named ICE-Fever) presenting a "TRAPS-like phenotype" (recurrent fever episodes, rash and arthralgia). Patients' mutations in pro-caspase-1 displayed a reduced enzymatic activity with an impaired cleavage of RIP-2, a protein regulating NFκ-B activation (83).

P. Ferguson (Iowa City, USA) presented a family affected with a variant of cherubism, characterized by some inflammatory features consistent with the autoinflammatory diseases. Cherubism is a rare autosomal dominant disorder due to mutations in the SH3 binding protein 2 gene (SH3BP2) (84). It usually presents with jaw enlargement that begins shortly after the primary teeth begin to erupt. Once all permanent teeth are present, the process often remits. The identified family presented a unique autoinflammatory phenotype including sterile, local pyogenic reaction following immunization, childhood onset nodular inflammatory rash, and, in one case, recurrent sterile osteomyelitis of the mandible; a phenotype which is distinct from both CRMO and cherubism. The skin and bone lesions were not responsive to antibiotics but improved with daily or every other day oral corticosteroids and bi-weekly interferon-alpha injections. A heterozygous SNP (H338P) was identified in exon 8 (c.1274A>C) in all affected individuals from this kindred. This non-synonymous SNP is not found in any of the SNP databases, is highly conserved and was not detected in >2000 control chromosomes, which supports the hypothesis that this is the causative mutation in this family (85).

A cohort of patients with the Nakajo-Nishimura syndrome (also called Familial Japanese Fever, MIM 256040) was presented by N. Kanazawa (Wakayama, Japan). It is an autosomal recessive condition characterized by an early onset of periodic spiking fever, cutaneous manifestations (pernio-like eruptions, nodular erythemas), bone deformities (clubbed fingers) and partial lipomusculodystrophy (86). It is characterized by a limited geographical distribution in the Wakayama district of Japan. A candidate gene approach is in progress to identify the possible associated gene (87).

Amyloidosis in autoinflammatory diseases

AA amyloidosis is a possible complication of chronic inflammatory or infectious disorders as well as of autoinflammatory diseases. It might occur at some stage if serum-amyloid protein A (SAA) is persistently elevated, but it does not necessarily reflect the clinical severity of the associated disease. Therapies that reduce SAA concentration are effective in treating amyloidosis.

P. Hawkins (London, UK) reported on his series of about 3000 cases of amyloidosis, 16% AA amyloidosis and 55% AL amyloidosis; AA amyloidosis associated with autoinflammatory diseases accounts for 9%, while 34% of cases are associated with rheumatoid arthritis. The mean time between disease onset and amyloidosis diagnosis is 23 years for AA amyloidosis secondary to autoinflammatory diseases. AA amyloidosis exhibits mainly renal involvement (nephrotic syndrome in 57% of cases, end-stage renal failure in 11%); liver and gastrointestinal system are involved only in late stages, heart and adrenal glands rarely, nervous system very occasionally. He illustrated diagnostic techniques (from histology to immunochemistry, to SAP-scintigraphy), and reviewed current treatments. SAA should be introduced in FMF monitoring, on the basis of correlations of SAA levels and survival and mortality. No differences were found between different dialysis modalities in AA amyloidosis induced renal failure; renal transplantation (recurrences

in 27% of the cases) provided a better organ and patient survival (88).

L. Obici (Pavia, Italy) reviewed new therapies in AA amyloidosis. She reported the encouraging results of phase II/III double-blind open-label extension studies with eprodisate, an oral small highly sulfonated molecule that acts by inhibiting the polymerization and deposition of amyloid fibrils. In the Eprodisate group, a 41% reduction in risk of disease worsening and a longer time to end-stage renal failure were observed (primary and secondary endpoints), along with a good safety profile (89). Experimental experiences with CHPC, a SAP binding inhibitor, in patients with hereditary fibrinogen amyloidosis, doxy-doxorubicin in transthyretine amyloidosis, doxycycline in transthyretine and β 2microglobulin amyloidosis were presented.

G. Hatemi (Istanbul, Turkey) conducted a retrospective study on 32 patients affected by secondary AA amyloidosis, treated with etanercept/infliximab for up to 7 years. Treatment with TNF- α antagonists over one year seems to stabilize proteinuria and reduce the increase in serum creatinine levels (90).

B. Balci-Peynircioglu (Ankara, Turkey) studied expression of ASC in renal tissues of familial Mediterranean fever patients with amyloidosis providing new insights on a possible role for ASC in AA type amyloid deposition (91).

Novel treatments for autoinflammatory diseases

Since the first demonstration of the dramatic efficacy of the IL-1 blockade with anakinra in cryopyrin-related disorders (92), a number of subsequent studies have reinforced the concept of the pivotal role of this therapeutic strategy not only in the classical monogenic autoinflammatory diseases, but also in other diseases such as systemic onset juvenile idiopathic arthritis (74, 76), adult Still's disease (93), Schnitzler's syndrome (77), gout (13, 14), steroid-dependent recurrent pericarditis (81). New IL-1 blockers are currently into experimentation. The efficacy and safety of an alternative IL-1 blocker, rilonacept in patients with FCAS has been mentioned above (23). Radin *et al.*

(Tarrytown, USA) reported on a placebo-controlled pilot study of rilonacept in 10 patients with refractory chronic active gouty arthritis. A significant reduction in subject's pain VAS score, severity of joint score and CRP was observed on rilonacept therapy, but not on placebo. No serious adverse events or opportunistic infections were reported (94).

The efficacy of the response to ACZ885 (a human IgG1 anti-IL-1 β monoclonal antibody) in patients with Muckle-Wells syndrome (MWS) was reported in two different studies.

JB Kuemmerle-Deschner (Tubingen, Germany) reported the experience on a phase II open-label study. In this study 12 MWS patients underwent a single ACZ885 s.c. injection (adults 150 mg, children 2 mg/kg) followed by an observation period and re-dosing upon recurrence of MWS symptoms. All 12 pts achieved complete and rapid clinical and serological response. Two children and 1 adult received i.v. injection to achieve complete response after s.c. administration. The median time to re-dosing was 92 days (9 pts) after the first and 66 days (6 pts) after the second treatment cycle (95).

H. Lachmann (London, UK) reported about the experience on a total of 8 MWS patients treated with ACZ885 intravenously (10mg/kg at the 1st dose and 1mg/kg for the following infusions) and then sub-cutaneously (150 mg) at the moment of disease relapses. An improvement in clinical symptoms within 1 day and complete clinical remission within 7 days together with the normalization of CRP and SAA was achieved in all 8 patients. The mean period of complete wellbeing after ACZ885 administration ranged from 90 to 186 days. The drug was uniformly well tolerated (96). A phase 3 randomized withdrawal placebo controlled study is currently underway.

P. Miettinen (Calgary, Canada) reported on the efficacy of IV pamidronate in 10 patients with chronic recurrent multifocal osteomyelitis (CRMO). After a 1st cycle of 3 days (0.5 mg/kg on day 1, 1 mg/kg on days 2-3) patients were treated with a subsequent single monthly infusion or with a complete 3-days cycle

every 3 months. A dramatic reduction of pain was observed in all treated patients. Moreover, the normalization of MRI abnormalities was achieved after a mean of 6.1 months (range 2-12). 4/10 patients relapsed after a mean of 12.3 months (range 9-18) after the first pamidronate infusion. Myalgia and fever were observed in four patients at the time of first infusion (97).

The Sixth International Congress on FMF and systemic autoinflammatory diseases will take place in Amsterdam, The Netherlands, 2-6 September, 2010 (<http://fmf.igh.cnrs.fr/ISSAID>).

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