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Review

Isoprenoid biosynthesis in hereditary periodic fever syndromes and inflammation

S. M. Houten [a,c,*], J. Frenkel [b] and H. R. Waterham [a]

[a] Laboratory Genetic Metabolic Diseases, Departments of Pediatrics/Emma Children's Hospital and Clinical Chemistry, Academic Medical Center, University of Amsterdam, Amsterdam (The Netherlands)
[b] Departments of General Pediatrics and Pediatric Immunology, Wilhelmina Children's Hospital, University Medical Center, Utrecht (The Netherlands)
[c] Present address: Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS/INSERM/ULP, BP 10142, 67404 Illkirch Cedex (France), Fax: + 33 3 88 65 32 01, e-mail: smhouten@igbmc.u-strasbg.fr

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Abstract. Mevalonate kinase (MK) is an essential enzyme in the isoprenoid biosynthesis pathway which produces numerous biomolecules (isoprenoids) involved in a variety of cellular processes. The indispensability of MK and isoprenoid biosynthesis for human health is demonstrated by the identification of its deficiency as the biochemical and molecular cause of the inherited autoinflammatory disorders mevalonic aciduria and hyperimmunoglobulinemia D and periodic fever syndrome. Since the discovery of the genetic defect, considerable progress has been made in understanding the molecular, biochemical and immunological basis of MK deficiency. Important questions such as which specific protein(s) and/or signaling pathway(s) are affected, however, remain unanswered. Resolving the complete pathophysiology of this disorder is a major challenge, but eventually will give insight into the in vivo role of MK and isoprenoid biosynthesis in inflammation and fever. This may open novel options for antiinflammatory therapies in general. Here, we give a general introduction on isoprenoid biosynthesis, the regulation thereof and deficiencies therein. We review the molecular, biochemical and immunological aspects of MK deficiency and discuss the relations between isoprenoid biosynthesis and inflammation. Finally, we compare MK deficiency with other autoinflammatory syndromes.

Key words. Isoprenoid biosynthesis; mevalonate kinase; hyper-IgD and periodic fever syndrome; mevalonic aciduria; autoinflammatory syndromes; inflammation; fever.

Introduction

Isoprenoids are a class of biomolecules that function in a wide variety of cellular processes. Mevalonate kinase (MK; EC 2.7.1.36) is an essential enzyme in isoprenoid biosynthesis. With the finding of the deficiency of this enzyme as the underlying defect in the disorders mevalonic aciduria (MA; MIM 251170) and hyperimmunoglobulinemia D (hyper-IgD) and periodic fever syndrome (HIDS; MIM 260920), it has become apparent that dysregulation of isoprenoid biosynthesis can lead to fever and inflammation. Therefore, MA and HIDS are classified as autoinflammatory disorders, a group of diseases characterized by spontaneous attacks of systemic inflammation without an apparent infectious or autoimmune etiology. In this review we will discuss recent developments in the relation between inflammation and iso-

* Corresponding author.

prenoids. First, we will give an overview of the isoprenoid biosynthetic pathway, its regulation and its deficiencies. Then we will focus on MK deficiency and our current understanding of its biochemical, molecular and immunological basis. With respect to the pathogenesis of this syndrome we will discuss the relation between MK deficiency, inflammation and other autoinflammatory syndromes.

Biosynthesis of isoprenoids

Isoprenoids make up a large group of essential molecules involved in diverse cellular processes. Among these are (i) ubiquinone-10, functioning as an antioxidant and involved in electron transport in the mitochondrial respiratory chain; (ii) heme A, present in the multiple heme-containing cytochrome c oxidase functioning in the mitochondrial respiratory chain; (iii) dolichol, a mediator of N-linked protein glycosylation; (iv) isopentenyl transfer RNAs (tRNAs), involved in protein translation and (v) isoprenylated proteins. Isoprenylation is the posttranslational covalent addition of farnesyl and geranylgeranyl moieties to proteins, which in most cases makes them membrane associated. Many isoprenylated proteins participate in important cellular functions, such as signal transduction, cell cycle control, cytoskeletal organization and intracellular vesicle trafficking [1].

In addition to these nonsterol isoprenoid compounds, cells produce sterols such as cholesterol. Cholesterol functions not only as a lipid in, for example, the lipid raft microdomains in cellular membranes [2], but also as the precursor for steroid hormones, bile acids and oxysterols. Furthermore, cholesterol can be linked covalently to the hedgehog class of embryonic signaling proteins, which function in embryonic tissue patterning [3]. All isoprenoids are composed of one or more (modified) C5 isoprene units. This C5 isoprene unit is synthesized in the mevalonate pathway from acetyl-coenzyme A (CoA).

The mevalonate pathway

In all metazoan organisms isoprenoids are made via the mevalonate pathway. This pathway starts with three molecules of acetyl-CoAs, which are converted into one molecule of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) in two enzyme steps (fig. 1) [4]. HMG-CoA is the substrate of the rate-limiting enzyme of the pathway, the highly regulated HMG-CoA reductase, which produces mevalonate [5]. Subsequently, mevalonate is phosphorylated twice, which yields 5-pyrophosphomevalonate. The first phosphorylation step is performed by MK, the enzyme deficient in MA and HIDS. The biochemical and molecular characterization of MK was reviewed recently

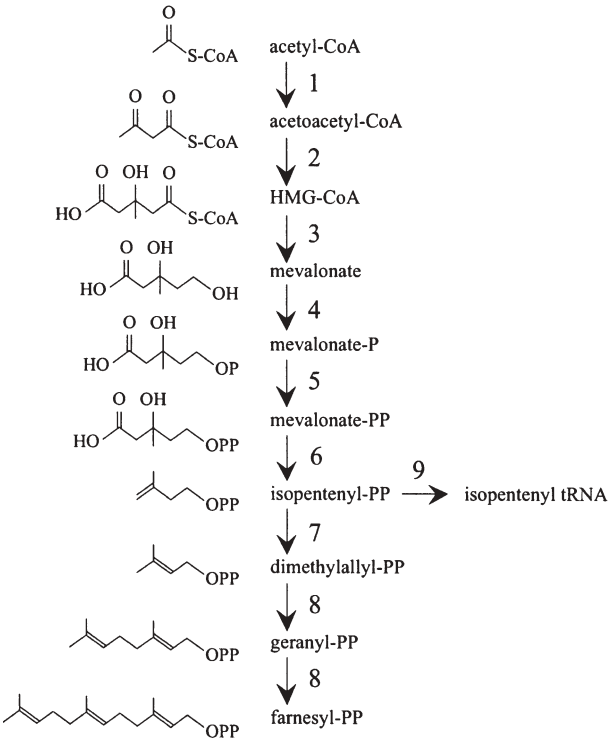

Figure 1. The mevalonate pathway. The figure presents the structures and names of the metabolites involved. The different enzymes involved are numbered as follows: (1) acetoacetyl-CoA thiolase (acetyl-CoA:C-acetyltransferase, EC 2.3.1.9); (2) 3-hydroxy-3-methylglutaryl-CoA synthase (EC 4.1.3.5); (3) 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase, EC 1.1.1.34); (4) mevalonate kinase (MK, EC 2.7.1.36); (5) phosphomevalonate kinase (EC 2.7.4.2); (6) mevalonate pyrophosphate decarboxylase (EC 4.1.1.33) (7) isopentenyl pyrophosphate isomerase (EC 5.3.3.2); (8) farnesyl pyrophosphate synthase (geranyltranstransferase, EC 2.5.1.10); (9) tRNA isopentenyltransferase (EC 2.5.1.8).

[6] and will be discussed only in relation to the recent elucidation of its crystal structure [7, 8].

Decarboxylation of 5-pyrophosphomevalonate gives isopentenyl pyrophosphate, the basic C5 isoprene unit. After isomerization to dimethylallyl pyrophosphate, a head-to-tail condensation of one molecule of isopentenyl pyrophosphate to one molecule of dimethylallyl pyrophosphate results in the formation of one molecule of geranyl pyrophosphate. Addition of another molecule of isopentenyl pyrophosphate gives farnesyl pyrophosphate (FPP), the branch-point metabolite. Another fate of isopentenyl pyrophosphate is its addition to an adenosine in some tRNAs (fig. 1).

The branch-point enzymes and cholesterol biosynthesis

FPP is the precursor for almost all isoprenoids and consequently substrate for all branch-point enzymes (fig. 2).

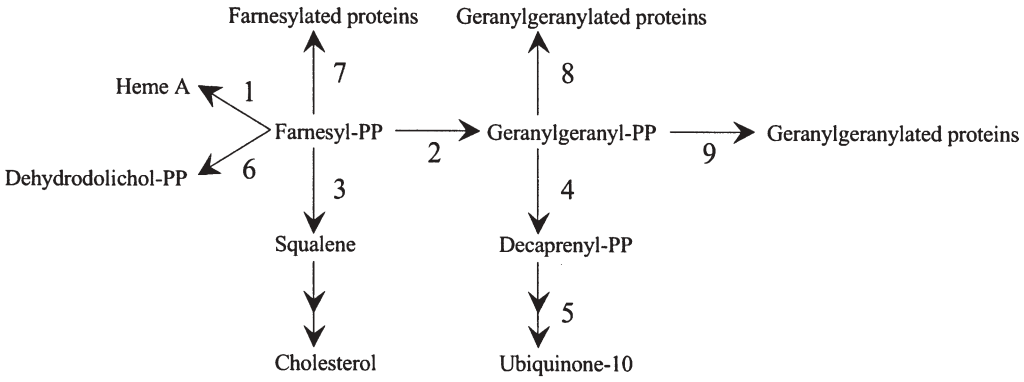

Figure 2. The branch-point enzymes. The figure presents end products, which can arise from the branch-point metabolite FPP. The different enzymes involved are numbered as follows: (1) heme A:farnesyltransferase; (2) geranylgeranyl pyrophosphate synthase (farnesyltranstransferase, EC 2.5.1.29); (3) squalene synthase (farnesyl diphosphate farnesyltransferase, EC 2.5.1.21); (4) decaprenyl pyrophosphate synthase (trans-nonaprenyltranstransferase, EC 2.5.1.11); (5) decaprenyl-4-hydroxybenzoate transferase; (6) dehydrodolichyl pyrophosphate synthase (cis-prenyltransferase); (7) protein farnesyltransferase; (8) protein geranylgeranyltransferase I; (9) protein geranylgeranyltransferase II.

The second substrate of these enzymes can be either a protein, heme A, isopentenyl pyrophosphate or FPP. Addition of one isopentenyl pyrophosphate to FPP gives geranylgeranyl pyrophosphate that can be used either directly for geranylgeranylation of proteins or further elongated to nonaprenyl or decaprenyl pyrophosphate. These latter molecules are used for the biosynthesis of ubiquinone-9 in rats and mice or ubiquinone-10 in humans, which contain 9 or 10 isoprene units, respectively. In contrast to these so-called trans-prenyltransferases or E-prenyltransferases, dehydrodolichol pyrophosphate synthase, that is involved in dolichol synthesis, is a cis-prenyltransferase or *Z*-prenyltransferase [9].

Three different protein prenyltransferases have been identified. Protein farnesyltransferase is related to protein geranylgeranyltransferase I: both are heterodimers sharing a common α subunit. The β subunits are different but share significant sequence similarity. Protein substrates for farnesyltransferase are Ras proteins and nuclear lamins, whereas geranylgeranyltransferase I uses Rac, Rho and most γ subunits of heterotrimeric G proteins. Geranylgeranyltransferase II is known also as Rab geranylgeranyltransferase, and geranylgeranylates proteins that belong to the Rab protein family [1].

Condensation and reduction of two molecules of FPP by squalene synthase yields squalene, a C30 molecule (composed of 6 isoprene units) and the first committed intermediate in the production of sterol isoprenoids (fig. 2). Cholesterol (C27) is produced via a complex set of enzymatic reactions [10–13].

Metabolism of farnesol and geranylgeraniol

Farnesol and geranylgeraniol are not direct intermediates in the biosynthesis of isoprenoids; however, both compounds can be utilized by cells for isoprenoid biosynthesis when added to the culture medium [14–16]. For example, farnesol and geranylgeraniol can rescue farnesylation and geranylgeranylation of proteins and cell cycle progression, when HMG-CoA reductase is inhibited by the addition of statins [17]. On the other hand, farnesol is an intermediate in the degradation of superfluous FPP and has been implicated to be a posttranscriptional, nonsterol regulator of HMG-CoA reductase since it accelerates the turnover of the HMG-CoA reductase protein [18–24]; however, this is still a matter of debate [25]. In addition, exogenous farnesol and/or geranylgeraniol have an effect on several other physiological processes, including inhibition of phosphatidylcholine biosynthesis [26, 27], induction of apoptosis, inhibition of cell cycle progression and actin cytoskeletal disorganization [26, 28].

Farnesol and geranylgeraniol cannot be used directly for isoprenoid biosynthesis, but have to be 'activated' to their pyrophosphates, FPP and geranylgeranyl pyrophosphate. The activating enzymes have been characterized at the biochemical level, and it was shown that farnesol and geranylgeraniol are phosphorylated by two successive monophosphorylation reactions [29]. The first step appears aspecific for added nucleotides, whereas the second step is performed by a CTP-specific kinase [29–31].

Degradation of FPP also occurs. This was noted when potent squalene synthase inhibitors (zaragozic acids) were isolated and characterized [32]. Like statins which inhibit HMG-CoA reductase, zaragozic acids could be useful as lipid-lowering drugs. When rats were fed with a zaragozic acid, they excreted several novel dicarboxylic acids in urine, identified as specific breakdown products of FPP (farnesol-derived dicarboxylic acids) [33]. In cells, excess FPP is converted into farnesol by farnesyl pyrophosphatase [22]. Farnesol is oxidized to farnesoic acid and in the liver via ω- and β-oxidation

converted into several dicarboxylic acids, which can be excreted in urine.

In vitro experiments in fibroblasts did not reveal activation of the alcohol precursors of isopentenyl pyrophosphate and dimethylallyl pyrophosphate (3-methyl-3-buten-1-ol and 3-methyl-2-buten-1-ol, respectively) [S. M. Houten and H. R. Waterham, unpublished observations]. This suggests a specific metabolism for farnesol and geranylgeraniol. The physiological relevance of these 'salvage' and 'degradation' pathways remains to be demonstrated.

Regulation of the mevalonate pathway

As is the case for most anabolic pathways, isoprenoid biosynthesis is regulated tightly in order to allow a constant production of the various isoprenoid molecules and to avoid overaccumulation of toxic intermediates or products, such as cholesterol [5]. Feedback regulation of isoprenoid biosynthesis by cholesterol is achieved predominantly through repression of transcription of genes that govern the synthesis of cholesterol (HMG-CoA synthase and HMG-CoA reductase), and its receptor-mediated uptake as plasma lipoproteins [low-density lipoprotein (LDL) receptor] [5]. This transcriptional regulation is performed by the so-called sterol regulatory element binding proteins (SREBPs; for reviews on this subject see [5, 34–39]). There is substantial evidence that most if not all enzymes of isoprenoid and cholesterol biosynthesis are under coordinate regulation by SREBPs, since overexpression of these proteins in mice induces expression of all studied enzymes involved in isoprenoid and cholesterol biosynthesis [40]. Furthermore, when rats were fed diets with bile acid sequestrants and/or statins, there was an increase in the activities of HMG-CoA reductase, MK, phosphomevalonate kinase and isopentenyl pyrophosphate isomerase [41–44].

The increase in HMG-CoA reductase activity can be much higher than expected from the increase in HMG-CoA reductase messenger RNA (mRNA) level. For example, statins, which are potent competitive inhibitors of the HMG-CoA reductase enzyme, can induce a 200-fold increase in HMG-CoA reductase protein, whereas mRNA levels are elevated only eightfold [5]. This is due to the multilevel regulation of this enzyme. In addition to the regulation at the transcriptional level, HMG-CoA reductase activity can be regulated also by two posttranscriptional mechanisms. These include translational efficiency of the HMG-CoA reductase mRNA and turnover of the HMG-CoA reductase protein [5]. The translation rate of HMG-CoA reductase mRNA is dictated by the cells' demand for nonsterol isoprenoids. When mevalonate production is blocked by statins, the HMG-CoA reductase mRNA is translated efficiently even in the presence of sterols, but when also the nonsterol requirements are satisfied by the addition of mevalonate, the translation rate reduces fivefold [45]. The degradation rate of the protein is governed by both sterol and nonsterol isoprenoids and can be accelerated fivefold [5, 46]. The sterols probably act via the membrane-spanning domain of HMG-CoA reductase, which is not necessary for catalytic activity, but also has a so-called 'cholesterol-sensing domain' [5]. Farnesol is most probably a nonsterol regulator of HMG-CoA reductase degradation [18–24]. Thus, the combined action of these two mechanisms together with the transcriptional regulation can induce an impressive adaptive response.

Disorders of isoprenoid biosynthesis

Most disorders of isoprene biosynthesis are caused by enzyme deficiencies in the post-squalene part of the pathway and affect only the biosynthesis of cholesterol. The syndromes include Smith-Lemli-Opitz syndrome (MIM 270400) [47, 48], desmosterolosis (MIM 602938) [49], X-linked dominant chondrodysplasia punctata (MIM 302960) [50–52], congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome (MIM 308050) [53, 54] and lathosterolosis [55]. In addition, cholesterol biosynthesis defects may be also the biochemical basis of hydrops-ectopic calcification-moth eaten skeletal dysplasia (MIM 215140) [56] and Antley-Bixler syndrome (*FGFR2*-negative patients; MIM 207410) [57] (for recent reviews on this topic, see [56, 58]). In line with the important role of cholesterol in embryogenesis and development, these syndromes are characterized by multiple congenital and developmental abnormalities. Other disorders affect the biosynthesis of heme A and ubiquinone-10 and cause respiratory chain deficiencies [59, 60]. Two isolated deficiencies in protein prenylation have been reported. Choroideremia (MIM 303100) is caused by mutations in the Rab escort protein, leading to hypogeranylgeranylation of one specific Rab protein (Rab27a). This leads to chorioretinal degeneration, invariably resulting in complete blindness. Griscelli syndrome (MIM 214450) is the second disorder in which protein prenylation is affected. The disease is caused by mutations in the RAB27A gene itself. In this case, this leads to immunodeficiency due to failure of cytotoxic T lymphocytes to secrete their lytic granules. Most patients develop a virus-induced hemophagocytic lymphohistiocytosis (T lymphocyte and macrophage activation syndrome) [61].

To date, MK deficiency is the only disorder identified that affects the biosynthesis of all isoprenoids since the defect occurs in an enzyme functioning early in the mevalonate pathway. Although two distinct clinical presentations have been defined, MA and HIDS, all patients

suffer from similar recurrent fever episodes and generalized inflammation. Therefore, the disease is considered an autoinflammatory disorder. MK deficiency will be discussed in more detail in the next paragraphs.

MK deficiency

Based primarily on clinical signs and the failure to recognize the large overlap (see below) two separate autosomal recessive entities have been reported to be caused by MK deficiency. However, after the identification of the underlying genetic basis for both defects, it is now clear that they simply represent the severe (MA) and mild end (HIDS) of a clinical and biochemical spectrum and consequently should be regarded as one defect, which we will call MK deficiency from here on. In the severe MA presentation, first reported in 1985 [62], MK enzyme activity is usually below detection levels when measured in cultured skin fibroblasts or lymphoblasts of patients [63, 64]. In the more benign presentation HIDS, however, a residual MK activity is measured which varies between 1 and 7% in cultured skin fibroblasts and peripheral blood mononuclear cells (PBMCs), and 2 and 28% in cultured lymphoblasts [65–67]. As a result of the different MK deficiencies, excretion of mevalonate in the form of mevalonic acid in urine differs markedly between the two syndromes. MA is characterized by a massive and constitutive excretion (1–56 mol/mol creatinine), which correlates with the severity of the clinical presentation [63]. In HIDS the excretion is moderate (0.005–0.040 mol/mol creatinine) and noted more readily during febrile crises [65]. Kelley reported that excretion in HIDS patients is at least 10-fold higher between fever episodes, and increases 100- to 500-fold above normal during febrile crises [68]. In control subjects the excretion of mevalonic acid in urine is usually less than 0.001 mol/mol creatinine.

The clinical distinction made between the two presentations involves the presence of congenital and developmental anomalies in patients with the MA presentation. Severely affected MA patients have profound developmental delay, dysmorphic features, cataracts, hepatosplenomegaly, lymphadenopathy, and anemia as well as diarrhea and malabsorption, and often die in infancy. Less severely affected patients have psychomotor retardation, hypotonia, myopathy and ataxia. The ataxia in these patients is probably due to a selective and progressive cerebellar atrophy [63]. However, like HIDS patients all MA patients also suffer from recurrent episodes of fever, which may occur up to 25 times per year and last on average 3–6 days. These fever episodes are associated with lymphadenopathy, arthralgia, subcutaneous edema, gastrointestinal problems, skin rash and sometimes death [63]. Several severely affected patients died during such crises. During a crisis there were no obvious metabolic derangements noted, only a highly increased blood sedimentation rate [63]. MA may often present as cholestatic liver disease and a hematologic disorder mimicking congenital infection, myelodysplastic syndrome or even acute leukemia in childhood [69, 70]. These hematologic abnormalities include a normocytic anemia with striking extramedullary hematopoiesis, hepatosplenomegaly, thrombocytopenia due to hypersplenism and leukocytosis with a left shift [69].

When compared with MA, HIDS is a relatively benign condition, in which patients mainly suffer from strikingly similar fever episodes, which occur every 2–6 weeks and last 3–7 days. Fever rises abruptly, often over 40 °C, and the temperature then gradually returns to normal. There is no strict periodicity, and patients may have long symptom-free periods. These fever episodes are associated with malaise, chills, headache, arthralgias, arthritis, nausea, abdominal pain, diarrhea, skin rash, hepatosplenomegaly and lymphadenopathy [71, 72]. The attacks in HIDS can be triggered by infections, minor trauma, childhood immunizations, menses or physical and emotional stress, but usually occur without any clear precipitating event [71]. In contrast to MA, HIDS patients usually have no or only few developmental features [71]. The first detailed description of the HIDS presentation was published in 1984 [73], and its name was derived from the constitutively elevated level of serum immunoglobulin (Ig)D, usually accompanied by elevated levels of serum IgA, which is still often used as a diagnostic hallmark of HIDS [71, 73], although less reliable and specific than MK enzyme activity measurements and/or mutation analysis of the gene encoding MK (MVK, see below). The disease has been reported also as very early onset juvenile arthritis and etiocholanolone fever [74, 75]. Most of the reported patients are of Dutch origin, and therefore the disease is also known as Dutch-type periodic fever. It should be noted, however, that periodic fever is a widely occurring phenomenon among children. The fact that the majority of currently identified HIDS patients are of Dutch origin may be the result of a heightened awareness of the disorder in the Netherlands and the inclusion of a specific laboratory test for IgD levels in Dutch patients with periodic fever.

With the availability of molecular analysis of the MVK gene and biochemical analysis of MK enzymatic activity in HIDS patients, it became apparent that not all patients clinically diagnosed with HIDS have MK deficiency [76, 77]. Consequently, patients with MK deficiency have been designated as having classic-type HIDS, whereas patients without MK deficiency has been denoted as having variant-type HIDS [77]. Upon closer inspection, however, subtle differences in symptoms, signs and laboratory findings were noted upon comparison of these HIDS variants. In general, patients with classic-type HIDS were

younger at the onset of the disease and had more additional symptoms during the fever episodes [76, 77].

Despite the increased knowledge about the genetic and biochemical basis, no effective treatment exists for MK deficiency at present. In a clinical trial with HIDS patients, thalidomide had only limited efficacy [78]. Leukotriene receptor inhibitors may reduce the severity of attacks in HIDS [79], suggesting that leukotrienes play a role in its pathophysiology. An enhanced urinary excretion of leukotriene E_4 was noted in MA patients, which strongly correlated with excretion of mevalonic acid in urine [80]. Leukotriene E_4 excretion in HIDS was elevated also, but only during fever episodes [81]. This increased excretion is the result of a higher systemic generation of cysteinyl leukotrienes, which appears to coincide with disease activity [80, 82].

Positive responses have been obtained also with corticosteroid treatment [63, 79]. A therapeutic trial in which two MA patients were treated with low doses of lovastatin in order to block the production of mevalonate (speculated to be pathogenic) was unsuccessful and had to be stopped because of the development of severe clinical crises [63]. As mentioned above, statins are potent competitive inhibitors of HMG-CoA reductase and are widely used to treat atherosclerosis and familial hypercholesterolemia. This drug blocks the synthesis of mevalonate and as a consequence lowers the endogenous synthesis of isoprenoids. The outcome of this trial strongly suggests that the recurrent fever episodes in MK deficiency are not caused by an excess of mevalonate but by a shortage of isoprenoid end products. The marked difference in urinary mevalonic acid excretion in HIDS and MA patients also argues against a causative role of mevalonate in the pathogenesis of the febrile crises. MA patients have much higher mevalonate levels, but fever episodes occur as frequently as in HIDS. Despite the negative experience with the use of statins in MA [63], the efficacy of the drug is now tested in a clinical trial with HIDS patients. On the other hand, end-product supplementation in MA also seems to have little if any beneficial effect [63]; however, long-term administration of ubiquinone and vitamin C and E appears to stabilize the clinical course and improve somatic and psychomotor development [79].

The molecular basis of MK deficiency

Since the isolation of the human complementary DNA (cDNA) encoding MK [83] and the identification of the genetic bases of MA [83] and HIDS [65, 84], many disease-causing mutations have been identified both at the cDNA and the genomic level. These include many missense mutations, but also nonsense mutations and insertions. In addition, two deletions at the cDNA level have been described, suggesting the occurrence of splice site mutations [6, 64–67, 76, 83–86]. By far the most frequently occurring mutation is the 1129G>A transition, which changes the valine at position 377 into an isoleucine (V377I). This mutation has been found exclusively in patients with the HIDS presentation. Although few patients homozygous for V377I have been reported [66, 76], most patients are compound heterozygotes for this mutation, and a second missense mutation that has been identified in patients with the HIDS as well as the MA presentation (803T>C/I268T and 59A>C/H20P) [65–67, 84, 86]. These findings strongly suggest that the 1129G>A mutation is responsible for the HIDS phenotype.

It should be noted that homozygotes for the V377I mutation are underrepresented. This is based on the fact that the distribution of the V377I mutation within the group of patients carrying MVK mutations is significantly different from the expected Hardy-Weinberg equilibrium principle distribution. In addition, we found the carrier frequency for the V377I mutation in the Dutch population to be 1:153 [87]. This predicts an incidence between 1 and 6 V377I homozygotes per year, which is far more than actually observed. Thus, homozygotes for V377I might have a milder phenotype of MK deficiency or have no disease phenotype at all [87].

Evidence for the disease-causing nature of the identified mutations was obtained by characterization of mutant proteins by immunoblotting of fibroblast lysates of patients using an MK-specific antibody and heterologous expression of the mutant proteins in *Escherichia coli* [64, 65, 83, 85, 86]. In addition, several amino acids important for the function, stability or catalytic activity of MK were identified previously by site-directed mutagenesis followed by characterization of the resulting recombinant protein [88–90] and, more recently, by the elucidation of the crystal structure [7, 8]. When combining these data, a prediction can be made about the effect of some specific mutations. Almost all characterized mutations result in MK proteins with markedly decreased enzyme activity when expressed in *E. coli* and often with markedly decreased levels in fibroblast lysates (H20P, T243I, L264F, L265P, I268T, V310M and A334T) [64, 85, 86]. Of these mutations, the A334T mutation is of interest because it is associated with a milder presentation of MA [85]. The resulting mutant protein is stable, but the recombinant enzyme has an elevated K_m for mevalonate [85]. This suggests a function of this region in the stabilization of mevalonate binding, which is confirmed by the crystal structure of MK [7, 8]. A similar effect is predicted for the T243I mutation based on the site-directed mutagenesis (T243A) and the crystal structure [8, 90]. In line with this, the patient carrying this mutation was less severely affected than other patients described previously [86, 91]. The H20P mutation is predicted to disturb the secondary structure of the protein, which could explain the observed

instability of the protein. The I268T mutation occurs in a helix that is important for dimerization of the protein. The amino acid substitution may weaken the dimerization interaction, making the protein less stable [8].

The consequence and disease-causing nature of the common V377I mutation has been debated. This mutant protein exhibits considerable residual activity when expressed in *E. coli* [65, 92], but is hardly detectable in fibroblast lysates of HIDS patients as shown by immunoblotting and enzymatic assay [65], despite normal MK mRNA levels [66]. When expressed in *E. coli* but also in patient's fibroblast lysates, the mutant protein has only modest kinetic differences in comparison with the WT enzyme (sixfold increase of the K_m for mevalonate) and displayed similar thermal inactivation as the WT protein [92]. Since in the crystal structure V377 is over 18 Å away from the active site, the conservative substitution by Ile is very unlikely to have an effect on the catalytic activity [8]. Moreover, the crystal structure predicts that V377 can be replaced by an isoleucine without affecting the protein structure or folding [8]. From these results the authors concluded that the V377I mutation was unlikely to be responsible for the observed depressed MK protein levels and catalytic activity in HIDS [8, 92]. However, our recent results with patient fibroblast lysates demonstrate that the V377I allele does not so much affect the catalytic activity but primarily the maturation of the mutant protein into an active enzyme. When patient fibroblasts harboring the V377I MVK allele are switched from 37 to 30 °C culturing temperature, the cells exhibit a marked and continuing increase in MK enzyme activity. However, when the same cells are cultured at 39 °C, MK activity decreases further. This implies that the V377I allele encodes a polypeptide that apparently is not capable of folding efficiently in the correct conformation at physiological or higher temperatures, which will lead to degradation [93]. These results together with the genetic evidence such as the occurrence of this specific mutation in the vast majority of HIDS patients [66, 67], the low carrier frequency [84, 87], the results of linkage analysis [84] and the autosomal recessive mode of inheritance unequivocally demonstrate that the V377I allele is the cause of the depressed MK activity in HIDS patients.

The biochemical basis of MK deficiency

Biochemical abnormalities related to isoprenoid biosynthesis observed in fibroblasts or lymphoblasts derived from patients with the MA presentation are a decreased (but still substantial) biosynthesis of cholesterol, dolichol, ubiquinone-10 and glycosylated macromolecules [94, 95]. On the other hand, protein prenylation of Ras and RhoA proteins was normal as determined by their presence in membranes [S. M. Houten and H. R. Waterham, unpublished observations], and also cholesterol biosynthesis can be entirely normal depending on the culture condition [94, 96]. Thus, MA and HIDS cells apparently are able to compensate for their defect in MK. This is possible because they have increased activity of HMG-CoA reductase and the LDL receptor pathway [94, 96]. HMG-CoA reductase, which converts HMG-CoA into mevalonate, is believed to perform the main rate-limiting step in isoprenoid biosynthesis and is among the most highly regulated enzymes in nature [5]. The increased activity of HMG-CoA reductase in MK-deficient cells is insuppressible by exogenous LDL cholesterol and was further upregulated under cholesterol-free culture conditions [96], suggesting that the high basal HMG-CoA reductase activity in MA cells is not due to a shortage of sterol end products. Accordingly, HMG-CoA reductase mRNA levels are normal in MA cells, indicating that the sterol-dependent SREBP pathway, involved in transcriptional regulation, is not activated [S. M. Houten and H. R. Waterham, unpublished observations]. The increased HMG-CoA reductase activity, however, was down-regulated when the MA cells were supplied with sterols, farnesol, geranylgeraniol or mevalonate [S. M. Houten and H. R. Waterham, unpublished observations]. This indicates that the regulation of the mevalonate pathway is still functional. The fact that HMG-CoA reductase mRNA levels are normal suggests that one of the non-sterol-dependent regulatory mechanisms causes the increase in HMG-CoA reductase activity. As discussed above, these mechanisms act posttranscriptionally and involve higher mRNA translation efficiency and decreased protein turnover.

It appears that MA and HIDS cells are able to compensate for reduced MK activity by elevating their intracellular mevalonate levels. This is illustrated by the fact that addition of extra mevalonate to the medium leads to a downregulation of HMG-CoA reductase activity in MA fibroblasts [S. M. Houten and H. R. Waterham, unpublished observations]. This implies that the elevated HMG-CoA reductase activity observed in MA fibroblasts mainly serves to compensate for the leakage of mevalonate from the cell. This is inevitable since a higher mevalonate concentration in the cell will lead to an increased leakage. Since HMG-CoA reductase is not elevated in HIDS fibroblasts, mevalonate leakage is only minimal in these cells, reflecting a lower level of intracellular mevalonate. In accordance with this, MA fibroblasts are more sensitive to inhibition of HMG-CoA reductase by simvastatin than HIDS fibroblasts, whereas HIDS fibroblasts are more sensitive to this inhibition than control fibroblasts as demonstrated by the variable accumulation of nonisoprenylated proteins in the cytosol after treatment of cells with different concentrations of statins [S. M. Houten and H. R. Waterham, unpublished observations]. Also, in MA patients mevalonic acid ex-

cretion is 100–1000-fold higher than in HIDS patients [63, 68].

Elevation of intracellular mevalonate concentrations promotes a 'normal' flux through the isoprenoid biosynthesis pathway when the following three conditions are met: (i) MK is not saturated with substrate (if MK was saturated, any elevation in mevalonate levels would have no effect); (ii) HMG-CoA reductase is able to generate mevalonate levels that are high enough for MK to function at a normal rate (HMG-CoA reductase has to compensate for the leakage of mevalonate from the cell); (iii) HMG-CoA reductase is not subject to noncompetitive product inhibition by mevalonate (this cannot be the case because HMG-CoA reductase is insensitive to any form of product inhibition [97]). These conditions imply a unique mechanism for the pathogenesis of a metabolic disorder (fig. 3). Normally, the pathogenesis may be caused by toxic accumulation of some intermediate or a shortage of end products. A shortage of end products may be due either to an accumulation of an intermediate, which is a noncompetitive inhibitor of the previous enzyme in the pathway, or a residual activity that is too small for keeping up with the pathway flux, resulting in saturation of the deficient enzyme with substrate. In the case of HIDS and MA, elevated mevalonate appears to compensate for the deficiency of MK under normal conditions (fig. 3b). However, a decrease in the residual MK activity in HIDS caused by increased temperature (e.g. fever) will have a marked impact [93] (fig. 3c). Since the steady-state levels of MK protein are very low, the enzyme approaches saturation. Thus, the relative increase in intracellular mevalonate level must be much higher than in controls in order to keep the pathway flux normal, which leads to a temporary decrease in the pathway flux and in the production of isoprenoids. This is reflected by a compensatory increase in HMG-CoA reductase activity, indicating that MK becomes progressively rate limiting [93]. A temporary decrease in the production of isoprenoids will affect especially high-turnover or newly synthesized isoprenoids, such as ubiquinone-10 in plasma, which is decreased in most MA patients [95], prenylated small G proteins like Rho [98], which are involved in multiple cellular processes such as signal transduction or cytoskeletal organization and the isoprenylated guanylate-binding proteins, which are synthesized in response to interferon-γ (IFN-γ) and lipopolysaccharide (LPS) [99]. It seems conceivable that this instant shortage of nonsterol isoprenoids is responsible for the proinflammatory phenotype of HIDS and MA. Thus, even minor elevations in temperature, due to exercise or infections could set off a chain of events, with MK becoming progressively rate limiting, leading to a temporary deficiency of antiinflammatory isoprenoids, followed by inflammation and fever. The elevation of mevalonate levels (through the increase of HMG-CoA reductase activity) eventually will lead to normal isoprenoid production again (fig. 3d). The activation of antiinflammatory pathways will lead to termination of inflammation and fever in HIDS and MA.

The observed differences in stability and temperature sensitivity of mutant MKs may explain why HIDS patients display episodic fever [93]. Since such fever episodes are prominent not only in HIDS but also in MA, the same may be true for MA. Indeed, the fact that mevalonate excretion in urine of both HIDS and MA patients correlates with disease severity points to a similar mechanism [63, 68]. Unfortunately, the extremely low and already hardly detectable MK activity levels in MA cells do not allow demonstration of a similar temperature-sensitive phenomenon as in HIDS cells. However, the finding that an increase in temperature does not only affect mutant MKs, but also wild-type MK activity renders it highly plausible [93]. Even a small additional decrease in MK activity in MA cells may have far reaching consequences, for two possible reasons. MK is even more saturated with substrate than in HIDS, thus an additional increase in mevalonate concentration has little effect. This may be the case when an MVK mutation results in reduced MK protein levels. In addition, HMG-CoA reductase already appears maximally induced to compensate for the MK deficiency. A further induction to establish even higher mevalonate levels may not be possible. This may be true for the A334T mutation that alters the affinity of the enzyme for its substrate mevalonate. These reasons could form an explanation for the reported fatal outcome of a fever episode in several MA patients [63].

The negative outcome of the therapeutic trial with lovastatin in two MA patients as described above, illustrates that not an excess of mevalonate itself is the pathogenic factor in MA, but a shortage of one the isoprenoid end products. In fact, it illustrates the importance of maintaining elevated mevalonate levels.

The difference in activity of HMG-CoA reductase also provides an explanation for the observed differences in mevalonate excretion between HIDS and MA. In MA, there appears to be a constitutive derepression of HMG-CoA reductase. This is reflected by the elevated reductase activity in fibroblasts and PBMCs from MA patients [93, 96] and the level of excreted mevalonate (>800 mmol/day), which greatly exceeds the level of normal whole body cholesterol biosynthesis (4 mmol/day, equivalent to 24 mmol of mevalonate/day) [63, 85]. In HIDS, HMG-CoA reductase activity may be derepressed mainly at the onset of and/or during a fever episode or only in tissues where the relative MK expression is low. This is illustrated by the fact that HMG-CoA reductase in HIDS fibroblasts is within the normal range, whereas in PBMCs it is elevated significantly. Experiments with multiple tissue RNA dot blots seem to confirm the hypothesis that the ratio between MK and HMG-CoA reductase expres-

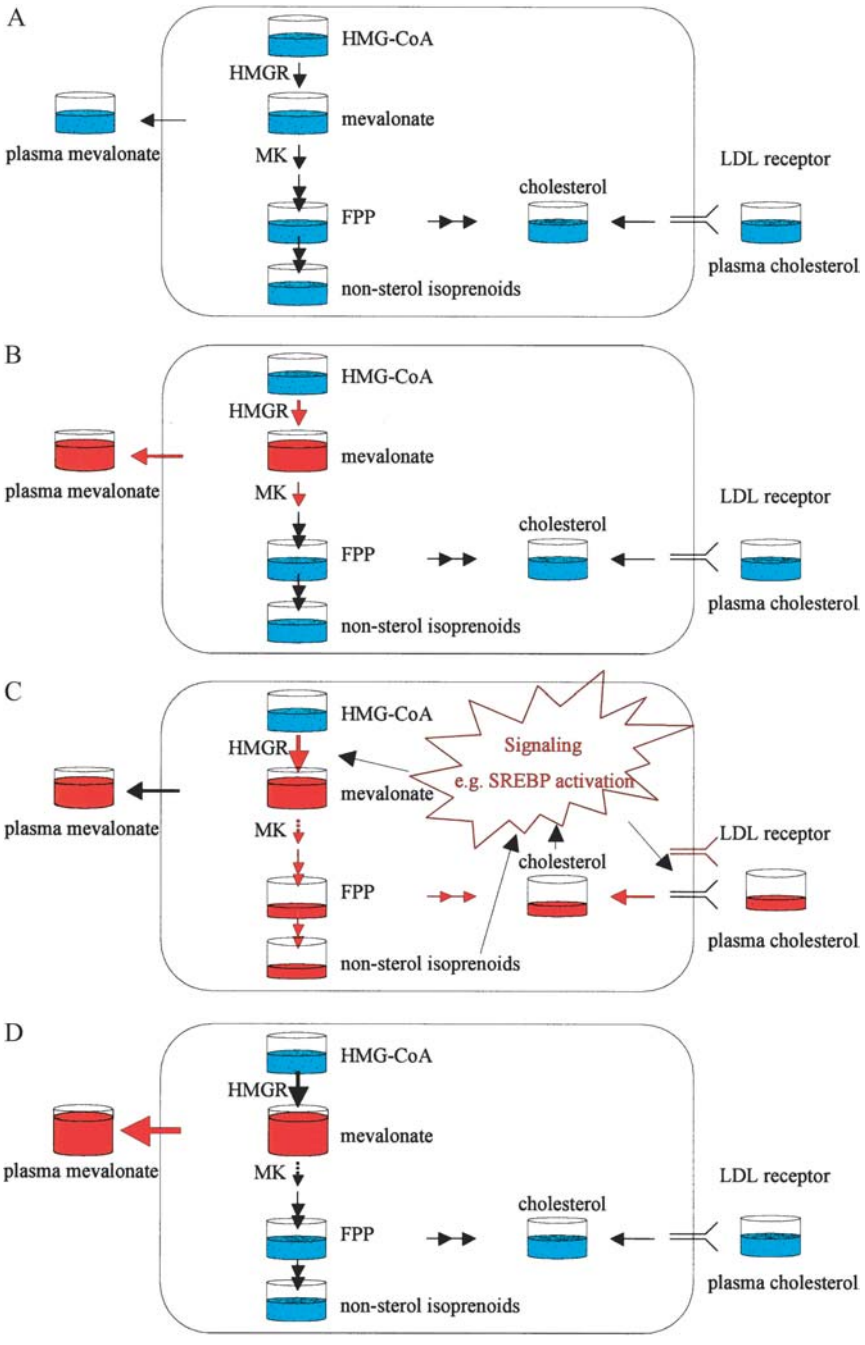

Figure 3. Schematic presentation of the biochemical basis of MK deficiency. (*A*) The steady state in isoprenoid biosynthesis in a normal cell. (*B*) The steady state in isoprenoid biosynthesis in an MK-deficient cell between a fever episode. (*C*) The dysregulation of isoprenoid biosynthesis during the development of fever and inflammation in an MK-deficient cell. (*D*) The novel steady state in an MK-deficient cell during a fever episode. The concentrations of different metabolites and end products are represented as reservoirs. Pathway flux is depicted with arrows. The control situation is displayed in blue, whereas any change or deviation from the control situation is displayed in red.

sion is variable and lowest in the appendix, bone marrow and peripheral blood leukocytes [S. M. Houten and H. R. Waterham, unpublished observations]. This also indicates that these tissues may be the most sensitive to lowered MK activity.

In accordance with the in vitro observations mentioned above, squalene, cholesterol and bile acid levels are usually (near) normal in patients with MA [63, 100]. In one of three patients tested, however, a decreased rate of biosynthesis of primary bile acids was noticed [101]. This again indicates that MA patients are capable of compensating for the reduced MK activity.

These results together with the above-described biochemical pathogenetic mechanism suggest that supplementation of isoprenoid precursors, such as mevalonate, farnesol and geranylgeraniol, may be beneficial in the abortion and prevention of fever episodes in HIDS and MA. However, the toxicity of these compounds has not been tested in vivo. In vitro, farnesol and geranylgeraniol have substantial cytotoxicity. Furthermore, they are able to downregulate HMG-CoA reductase enzyme activity. Since MK-deficient cells depend on an elevated HMG-CoA reductase activity, studies toward the in vivo effects of isoprenoid precursor supplementation are necessary.

The immunological basis of MK deficiency

Whereas studies on the pathogenesis of MA have mainly focused on biochemical aspects, studies on HIDS pathophysiology were focused on the dysregulation of the immune system observed in these patients. There are several hematological abnormalities observed during a fever attack, which are indicative of an acute phase response, including granulocytosis, increased erythrocyte sedimentation rate and elevation of several acute phase proteins such as C-reactive protein, serum amyloid A, fibrinogen, soluble type II phospholipase A_2 and α_1-acid glycoprotein [71]. The concentration of α_1-acid glycoprotein is increased continuously during attacks and remissions, as is the glycosylation of α_1-acid glycoprotein, indicating a persistent state of inflammation [102].

As mentioned above, in HIDS there is a constitutive polyclonal elevation of serum IgD, usually with concomitant high levels of IgA (subclass IgA1) [103]. An elevated serum IgD and IgA has been reported also in MA [67, 103]. Plasma cells residing in the bone marrow are probably the source of both the IgA and IgD, suggesting continuous, systemic stimulation of the immune system [104]. The physiological role of IgD and its role in the pathogenesis of HIDS, however, remains enigmatic. Although IgD is a potent inducer of cytokines in vitro [105], its elevation is more likely to be an epiphenomenon than the cause of the inflammatory state in HIDS, since there is no relation between IgD level and severity of the attacks, frequency of the attacks or disease activity itself. Accordingly, fever attacks in children may precede the rise in IgD [106]. Recently, we reported a patient with all the signs and symptoms of HIDS and deficient MK activity, but no serum IgD elevation. Serum IgA, however, was elevated in this patient [65]. Furthermore, IgD may be elevated in other autoinflammatory diseases, and various other diseases and conditions including infections, immunodeficiencies, autoimmune diseases, allergic diseases and malignancies [107].

The production of pro- and antiinflammatory cytokines in HIDS has been studied both in vivo in plasma from patients and in vitro in the culture supernatant of whole blood or PBMC cultures [108–110]. During febrile attacks the serum levels of IFN-γ and interleukin (IL)-6 rise sharply [108, 109], and tumor necrosis factor-α (TNF-α) rises to high normal values, whereas IL-1α and IL-1β are not elevated [109]. The effect of the increased stimulation of mononuclear phagocytes by IFN-γ is reflected in a rise in urinary neopterin excretion simultaneously with the onset of fever. The neopterin excretion remains high for several days after normalization of the body temperature [108]. Of the tested antiinflammatory cytokines, IL-1 receptor antagonist and soluble receptors for TNF-α (p55 and p75) were elevated, whereas IL-10 remained normal [109].

In supernatants of unstimulated cultures of whole blood samples of HIDS patients obtained between or during attacks, IL-1β and IL-6 are not increased [109]. Although plasma levels of TNF-α stay within normal limits, its concentration is increased in the supernatants of unstimulated cultures of whole blood samples drawn between attacks. IL-1 receptor antagonist was elevated only when blood was obtained during fever. When stimulated with LPS, the supernatants of such cultures showed an elevated TNF-α level, which was even higher when the blood cells had been obtained during an attack. IL-1β and IL-1 receptor antagonists were only elevated in these stimulated cultures when blood was drawn during an attack [109]. Similar results were obtained in culture supernatants of isolated PBMCs obtained between attacks. Notably, spontaneous IL-1β, IL-6 and TNF-α production by isolated PBMCs is elevated significantly in HIDS and rises further upon stimulation with LPS [110].

Taken together, these data are compatible with macrophage activation during the febrile attacks [72]. This is reflected by an increased expression of CD64 (Fcγ-receptor I) on monocytes and granulocytes during fever [J. Frenkel, unpublished observations]. Between the febrile attacks the in vitro findings are still compatible with increased activity of the mononuclear phagocytic compartment [72]. The cause of this macrophage activation is unknown. While activated Th1 cells are known to induce macrophage activation, the high IgD and IgA levels are more compatible with increased activity of Th2

cells [111]. However, direct evidence of T-cell activation during attacks is lacking [72]. A potential role for natural killer (NK) cells in the initiation of macrophage activation has not been addressed to date.

The relations between isoprenoid biosynthesis and inflammation

Besides the occurrence of a defect in isoprenoid biosynthesis in a syndrome characterized by dysregulation of the inflammatory response, there are several other indications that isoprenoid biosynthesis plays a role in inflammation and can influence the immune system.

Screening of neutrophil-derived lipid extracts revealed that presqualene diphosphate is a potent inhibitor of superoxide anion production (O_2^-) [112]. Presqualene diphosphate is formed by the condensation of two molecules of FPP by the enzyme squalene synthase. The same enzyme also catalyses the reductive rearrangement of presqualene diphosphate to squalene, which requires NADPH (nicotinamide adenine dinucleotide phosphate) [113]. It is conceivable that the oxidative burst in neutrophils that consumes NADPH also, limits the conversion of presqualene diphosphate to squalene. This would negatively regulate the production O_2^- during the oxidative burst.

It has been reported that administration of LPS, TNF-α or IL-1β to Syrian hamsters triggers a rapid upregulation of hepatic HMG-CoA reductase and a downregulation of squalene synthase, the enzyme catalyzing the first committed enzyme step of sterol biosynthesis [114–116]. The reduced squalene synthase activity may explain the observation that the rate of hepatic cholesterol synthesis increases by only twofold following LPS administration, despite a fourfold increase in HMG-CoA reductase activity [114]. In addition, these observations suggest that FPP preferentially flows into the nonsterol isoprenoids during inflammation.

Recently, the so-called pleiotropic or cholesterol-independent effects of statins received much attention (for recent reviews on this subject, see [117–120]). It has become clear that not all beneficial effects of treatment of atherosclerosis and familial hypercholesterolemia with statins could be due to lipid lowering, since some of these effects already occur very early in clinical trials of statin therapy [121, 122]. Since statins inhibit HMG-CoA reductase, which performs an enzyme step early in isoprenoid biosynthesis before the branch-point metabolite FPP, it is obvious that mevalonate depletion in principle cannot effect cholesterol biosynthesis only, but also the de novo synthesis of all other isoprenoids. Due to differences in the regulation and kinetic properties of the branch-point enzymes, however, there are differential effect of statin treatment on the different isoprenoid end products [123, 124]. Isoprenylation appears to be 100 times less sensitive to inhibition of mevalonate biosynthesis than is the synthesis of cholesterol [123].

Many antiinflammatory effects of statins have been reported, including the reduction of lymphocyte proliferation, and of the expression of major histocompatibility complex (MHC) class II molecules, matrix metalloproteinases, cytokines and chemokines (for a recent review on this subject, see [120]). On the other hand, these compounds also have proinflammatory properties. Statins enhance endothelial expression of cellular adhesion molecules [125]. Interestingly, the secretion of IL-1β, IFN-γ and IL-18 by PBMC stimulated in vitro with inactivated *Mycobacterium tuberculosis* is augmented greatly by the inhibition of isoprenoid biosynthesis with statins [126]. The increased cytokine secretion was due to lack of isoprenoids, since addition of mevalonic acid reduced cytokine secretion to control levels [126]. The same phenomenon was noted when purified monocytes were used. Treatment of these cells with statins increased the production of IL-1β, TNF-α, macrophage chemotactic protein-1 and IL-8 [127]. In addition, statins enhanced infiltration of mouse leukocytes into an inflamed peritoneal cavity [127]. A similar mechanism was shown to cause elevated cytokine secretion in HIDS and MA PBMC cultures [128]. Statins augmented IL-1β secretion by control stimulated PBMCs, which could be countered by bypassing HMG-CoA reductase with mevalonate, and, to a lesser extent, by farnesol or geranylgeraniol. In the absence of statins, mevalonate itself did not change the already minimal IL-1β secretion of PBMC, indicating that mevalonate by itself does not induce cytokine secretion. As reported before, HIDS and MA PBMCs spontaneously secrete more IL-1β than control PBMCs. This secretion increased even further in the presence of statins. Bypassing HMG-CoA reductase with mevalonate, farnesol or geranylgeraniol tended to abort this increased IL-1β secretion. More important, in the absence of statins, IL-1β secretion by PBMCs from HIDS and MA patients was reduced when cultured in the presence of farnesol [128]. These observations again indicate that not mevalonate but a shortage of one of the nonsterol end products is the pathogenic factor in HIDS and MA.

The other autoinflammatory syndromes

MK deficiency has been classified as an autoinflammatory (or noninfectious inflammatory) disorder [129–131], and as such belongs to a large group of diseases including systemic-onset juvenile chronic arthritis (MIM 604302), adult-onset Still's disease, periodic fever, aphtous stomatitis, pharyngitis and adenopathy (PFAPA) syndrome, Crohn's disease (inflammatory bowel disease, MIM 266600), Blau's syndrome (MIM 186580), Behçet's

syndrome (MIM 109650), familial Mediterranean fever (FMF, MIM 249100), TNF-receptor-associated periodic syndromes (TRAPS, MIM 142680) and the cryopyrin-associated periodic syndromes (CAPS), including familial cold auto-inflammatory syndrome (FCAS, MIM 120100) [132], Muckle-Wells syndrome (MWS, MIM 191900), and the chronic infantile neurologic cutaneous arthropathy (CINCA) syndrome (MIM 607115) [133]. All these diseases are characterized by spontaneous attacks of systemic inflammation without an apparent infectious or autoimmune etiology [129]. A subgroup of these diseases are the hereditary periodic fever syndromes, which include MK deficiency, FMF, TRAPS and CAPS [129, 130]. Although these diseases differ in some clinical aspects (see table 1), they share susceptibility to episodic severe generalized nonspecific inflammation. For most of these syndromes the molecular basis has been solved. The picture emerging is that all affected genes encode proteins involved in nonspecific inflammation. In the case of TRAPS, the receptor for TNF-α and lymphotoxin, TNFRSF1A is affected. Mutations may often impair shedding of the extracellular part of the receptor, leading to a reduction of the (antiinflammatory) soluble TNF receptor [134]. In FMF the affected gene (MEFV) encodes pyrin, also known as marenostrin [135, 136]. Pyrin is expressed exclusively in phagocytic cells, and its function is understood only partly. Recently, pyrin was shown to share a domain with many other proteins mainly involved in apoptosis and inflammation. This domain has been called pyrin domain and belongs to the six-helix bundle death domain-fold superfamily [137–140]. Cryopyrin, the gene affected in CAPS, contains a similar domain and is expressed also in phagocytes [141]. Members of the death domain-fold superfamily are well established mediators of protein-protein interactions. Likewise, pyrin domain-containing proteins interact with each other, and some members of this protein family take part in the formation of a signaling complex called the inflammasome [142, 143]. One of the main functions of the inflammasome is the activation of caspase-1 and processing of IL-1β and IL-18. It seems conceivable that other pyrin domain-containing proteins, notably pyrin and cryopyrin,

Table 1. Comparison between the hereditary periodic fever syndromes.

Syndrome	Hyper-IgD syndrome/ mevalonic aciduria	Familial Mediterranean fever	TRAPS	FCAS/MWS/CINCA-syndrome (CAPS)
Inheritance	Autosomal recessive	autosomal recessive	autosomal dominant	autosomal dominant
Gene	MVK	MEFV	TNFRSF1A	CIAS1
Protein	MK	marenostrin / pyrin	TNFRSF1A	cryopyrin
Function	isoprenoid biosynthesis	unknown	TNF receptor	unknown
Expression	ubiquitous	neutrophils	ubiquitous	neutrophils/chondrocytes
Typical ethnic origin	Dutch, French, other	Jews, Arabs, Turks, Armenians	British, Irish, other	worldwide
Typical age at onset	infancy	childhood	variable	neonatal/infancy
Typical duration of attacks	3–5 days	12–72 h	days to weeks	<24 h
Interval between attacks	weeks to months	weeks to months	weeks to months	variable
Vomiting	+	–	–	–
Diarrhea	+	–	+	–
Abdominal pain	+	++	+	–
Peritonitis	–	++	+	–
Pleuritis	–	+	+	–
Scrotal pain	–	infrequent	++	–
Skin involvement	++	rare	++	++
Ocular involvement	–	–	++	++
Ear involvement	–	–	–	+
Oropharyngeal involvement	+	–	–	–
Joint involvement	arthralgias, oligoarthritis	monoarthritis	arthralgias	arthralgias, CINCA: destructive arthritis of large joints
Headache	+	–	++	+, CINCA: aseptic meningitis
Mental retardation	MA: +	–	–	CINCA: +
Myalgias	+/– (MA: +)	infrequent	++	+
Lymphadenopathy	++	–	+	–
Splenomegaly	+	–	+	–
Amyloidosis	–	+	+	+ (MWS ++)
References	[63, 65, 83, 84]	[135, 136]	[134]	[132, 133, 144]

+, present; ++ frequent; – absent.

may act in an antiinflammatory manner by interfering with inflammasome assembly.

It will be interesting to study how the functions of the gene products defective in the other syndromes are related to the function of MK [131]. Currently, there is little information on the influence of MK deficiency on the quantity, localization or activity of these proteins. However, inhibition of isoprenoid biosynthesis by statins has been shown to raise IL-1β secretion [127] in a caspase-1-dependent fashion [126]. A similar mechanism seems to occur in MK deficiency [128]. The nature of the isoprenoid product affecting caspase-1 function, however, is unknown. It may well be possible that as in the other hereditary periodic fever syndromes, MK deficiency results in a predisposition to inflammatory attacks by interfering with normal inflammasome functioning.

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