IL-1 and mevalonate kinase deficiency
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General Introduction
The isoprenoid/cholesterol biosynthesis pathway

Isoprenoids are a class of biomolecules that function in a wide variety of cellular processes. These include among others; 1) the side chains of heme A and ubiquinone-10, both involved in electron transport in the mitochondrial respiratory chain, 2) dolichol, involved in N-linked protein glycosylation, 3) isopentenyl tRNA, used in protein translation and 4) the farnesyl and geranylgeranylation moieties of isoprenylated proteins that are involved in cell proliferation and differentiation (1). In addition to the synthesis of these nonsterol compounds, the isoprenoid biosynthetic pathway also produces sterol isoprenoids such as cholesterol. Cholesterol is an important structural component of cellular membranes and myelin, precursor of oxysterols, steroid hormones and bile acids, and plays an important role in human embryogenesis and development (2).

Isoprenoid biosynthesis starts with 3 acetyl-CoAs, which in two enzymatic steps are converted into 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)(figure 1). The reduction of HMG-CoA is catalyzed by the rate-limiting enzyme of the pathway, HMG-CoA reductase and yields mevalonate. Subsequently, mevalonate is phosphorylated twice, which produces mevalonate-5-pyrophosphate. This compound is decarboxylated into isopentenyl pyrophosphate (IPP, C5 unit). This 5-carbon unit is used for the synthesis of all isoprenoid end products including some tRNAs in which IPP is added to adenosine. IPP is isomerized into dimethylallyl pyrophosphate (DMAPP) followed by a head-to-tail condensation of IPP to DMAPP to form geranylpyrophosphate (GPP, C10 unit). Addition of another IPP gives farnesyl pyrophosphate (FPP, C15 unit), which is the branchpoint metabolite of the pathway and the precursor for almost all isoprenoids. Addition of again IPP to FPP produces geranylgeranylpyrophosphate (GGPP, C20 unit) that can either be used directly for geranylgeranylation of proteins or is further elongated to nonaprenyl or decaprenylpyrophosphate. The latter is used for the biosynthesis of ubiquinone-10. FPP is also the substrate for the synthesis of dehydrodolichol pyrophosphate. The enzymes responsible for isoprenoid addition to proteins comprise three distinct protein prenyltransferases classified in two functional classes: the CAAX prenyltransferases, including protein farnesyltransferase (FTase) and protein geranylgeranyltransferase type I (GGTase-I), and the Rab geranylgeranyltransferase or protein geranylgeranyltransferase type II (GGTase-II). FTase and GGTase-I are designated CAAX prenyltransferases because they recognize their protein substrates by their C-terminal CAAX sequence. Substrates for FTase include Ras GTPases, lamin B and transducin γ subunits, whereas substrates for GGTase-I include Rho, Rac and most γ-subunits of heterotrimeric G proteins. GGTase-II attaches geranylgeranyl groups to two C-terminal cysteines in GTPases of the Rab family, which have C-terminal Cys-Cys (CC) or Cys-X-Cys (CXC) motifs (3).

The conversion of two FPP molecules into squalene is the first reaction exclusively committed to the synthesis of sterol endproducts. This reaction is catalyzed by squalene synthase and is followed by the condensation of squalene to yield lanosterol. For the generation of C27 cholesterol from lanosterol, a series of at least 8 different enzyme reactions is required. These include one methylation at C14 and two demethylations at C4, three reductions of the Δ14, Δ7 and Δ24 double bonds, one isomerization of Δ8 to Δ7 and one desaturation between C-5 and C-6. Because most of
the cholesterogenic enzymes are rather non-specific and can handle various intermediates, their sequence may vary dependent on the tissue in which they occur. Consequently, two major routes involving the same enzymes are proposed, which, depending on the timing of the reduction of $\Delta^{24}$ double bond, postulate either 7-dehydrocholesterol or desmosterol as the ultimate precursor for cholesterol (4).

Figure 1: The isoprenoid biosynthesis pathway. Schematic overview of the isoprenoid biosynthesis pathway, indicating the enzyme mevalonate kinase, which is deficient in MKD.
Mevalonate kinase

Mevalonate kinase (MK) is the first enzyme to follow the highly regulated HMG-CoA reductase and catalyzes the phosphorylation of mevalonate to produce phosphomevalonate. In 1956, mevalonate was reported for the first time to be important in the biosynthesis of cholesterol (5). This intermediate was biologically active in its R-enantiomer isoform and it required cofactors as adenosine triphosphate (ATP), Mg\(^{2+}\) or Mn\(^{2+}\) and reduced pyridine nucleotides for the conversion to squalene (6;7). The need for ATP suggested the formation of a phosphorylated intermediate, which was later confirmed by the identification of 5-phosphomevalonate as the first product of the phosphorylation of mevalonate catalyzed by the enzyme accordingly called MK (8).

Kinetic analysis of the phosphorylation of mevalonate by MK has shown that first mevalonate associates with the enzyme, followed by the addition of MgATP. 5-Phosphomevalonate is released as first and ADP as second product (9).

Regulation of the isoprenoid biosynthesis pathway

To ensure a constant production of non-sterol isoprenoids and to avoid overaccumulation of endproducts such as cholesterol, the isoprenoid biosynthesis is tightly regulated. HMG-CoA reductase is the rate-limiting enzyme of the isoprenoid biosynthesis pathway and thus plays a central role in this regulation, which is controlled by multiple mechanisms. At the transcription level, a feedback mechanism in response to sterol levels is mediated by sterol regulatory element binding proteins (SREBPs). Mammalian cells express three isoforms of SREBP: SREBP-1a, -1c and 2. SREBP-1a-and –1c are produced from the same gene through alternative splicing. Each SREBP consist of an N-terminal transcription factor domain, a middle domain containing two hydrophobic transmembrane segments and a C-terminal regulatory domain (10). The two membrane-spanning helices anchor SREBPs in the membranes of the endoplasmatic reticulum (ER) and nuclear envelop (11) where they are tightly associated with the SREBP-cleavage-activating protein (SCAP).

Retention in the ER of the SREBP-SCAP complex requires interaction between the sterol sensing domain of SCAP and Insig-1 or -2, two membrane proteins of the ER (12-14). In the absence of sterols, SCAP and the Insig proteins do not interact and the SREBP-SCAP complex will be packaged into COPII vesicles and travel to the Golgi apparatus. In this compartment the N-terminal transcription factor domain of SREBP is released by two subsequent cleavages by the proteases called Site-1 and Site-2 protease (S1P and S2P) (15;16). The domain will then translocate to the nucleus where it activates transcription by binding to sterol regulatory elements in the promoter/enhancer regions of multiple target genes, including HMG-CoA reductase, the LDL receptor gene (which supplies cholesterol through receptor-mediated endocytosis) as well as many other genes involved in isoprenoid/cholesterol biosynthesis (17;18).

HMG-CoA reductase is also embedded in the ER membrane and contains a sterol-sensing domain. In the presence of cholesterol this domain binds one of the Insigs whereupon HMG-CoA reductase is ubiquinated and degraded (19). The degradation rate is not only governed by sterol isoprenoids but can also be accelerated by non
sterol isoprenoid, including derivatives of farnesyl pyrophosphate (e.g. farnesol) and geranylgeranylpyrophosphate (19-21). The translation rate of HMG CoA reductase is dictated by the cells' demand for non-sterol isoprenoids. When mevalonate production is inhibited by blocking HMG-CoA reductase with statins, the translation of HMG-CoA reductase mRNA is efficiently even in the presence of sterols. But when also the non-sterol requirements are satisfied by the addition of mevalonate, the translation rate reduces five-fold (22). HMG CoA reductase catalytic activity can also be regulated by the cellular energy state (ATP/AMP ratio). ATP depletion and consequently AMP rise cause the activation of the AMP-dependent kinase which in its turn decreases HMG CoA reductase activity via phosphorylation (23).

**Regulation of MK**

MK is one of the enzymes of the isoprenoid biosynthesis pathway that can be upregulated by SREBPs. The promoter region of the *MVK* gene, which encodes MK, contains two Sp1-binding sites and an SRE. Sp1 binding sites are the targets for the general transcription factor Sp1, whereas SREs enhance transcription by binding of SREBPs. The SRE in the *MVK* promoter has a 7/8 base pair match to the consensus sequence identified in the promoter of the HMG-CoA synthase, HMG-CoA reductase and the LDL receptor gene (24). Both MK and HMG-CoA reductase activity can be increased by inhibition of HMG-CoA reductase by statins although HMG-CoA reductase activity is induced 15-fold higher than MK activity. HMG-CoA reductase acts as the rate-limiting enzyme in this case although the rate-limiting step can shift to the MK when the MK enzyme is deficient, which is the case in patients with the metabolic defect mevalonate kinase deficiency. The mutated *MVK* gene in these patients still responds to feedback regulation by SREBPs ((25), chapter 2).

**Localization of the isoprenoid biosynthesis pathway**

Isopenoid biosynthesis takes place in the ER and cytosol. Enzymes with soluble substrates (i.e. enzymes in the pre-squalene part of the pathway) reside in the cytosol whereas the enzymes handling lipophilic substrates (post-squalene/cholesterogenic) are present in the ER membrane. The only exception to this is HMG-CoA reductase, which is an integral membrane protein of the ER, but has its catalytic domain localized in the cytosol (1). In addition, few of the post-squalene enzymes are also found in the nuclear membrane. Since the beginning of the eighties, several reports have appeared postulating a peroxisomal localization of the enzymes involved in the pre-squalene part of the pathway (reviewed in (26)). More recent studies have questioned the experimental approaches upon which this postulation was based (1;26-28). Experiments towards subcellular localization of endogenous proteins, which were performed under physiological conditions, revealed that there is little, if any, evidence for a direct role of peroxisomes in isoprenoid biosynthesis (27;29;30).
Localization of MK

The amino acid sequence of MK harbours an N-terminal stretch, which shares similarity with the consensus sequence of the peroxisomal targeting sequence type 2 (PTS2) and which has been implicated to be involved in peroxisomal targeting of MK via PTS2 receptor protein PEX7 (31). However, Ghys et al could not confirm a physical interaction between MK and PEX7; the human PTS2 receptor was not retained by Ni-NTA resin loaded with (His)$_6$-MK (32). Moreover, also no interaction between the two proteins was observed in a yeast two hybrid system (27). Recent data provide strong evidence for a cytosolic localization of MK rather than a peroxisomal localization (27).

Disorders of the isoprenoid biosynthesis pathway

The identification of a number of inherited disorders due to a defect in cholesterol biosynthesis has made clear that cholesterol plays a crucial role in human embryogenesis and development. Patients afflicted with these disorders are characterized by multiple morphogenic and congenital anomalies including internal organ, skeletal and/or skin abnormalities. Although the genetic causes underlying these defects are known, the etiology of the pathophysiology associated with the defects remains unclear and may involve multiple affected processes due to the lowered cholesterol and/or elevated levels of intermediate sterol precursors.

Most common defect of the cholesterol biosynthesis is the autosomal recessive Smith-Lemli-Opitz syndrome (SLOS; MIM 270400) (33), a multiple malformation syndrome caused by 3β-hydroxysterol Δ7-reductase deficiency (34). Desmosterolosis (MIM 602398) is a very rare autosomal recessive disorder of cholesterol biosynthesis for which only two patients are described who had similar clinical presentations as patients with SLOS (35;36). In both patients, mutations were found in the gene encoding 3β-hydroxy Δ24-reductase (37). In 2002, two patients with lathosterolosis (MIM 607330) were described, which is due to mutations in the gene coding for 3β-hydroxysterol Δ5-desaturase (38).

The two X linked dominant inherited disorders of cholesterol biosynthesis Conradi-Hünermann-Happle syndrome (CDPX2; MIM 302960) and CHILD syndrome (MIM 308050) are caused by deficiencies of sterol Δ8-Δ7 isomerase (39;40) and/or a sterol C-4 demethylase (41), respectively due to mutations in the encoding genes. Patients with CDPX2 or CHILD syndrome have similar presentations, including skeletal and skin abnormalities, among which chondrodysplasia punctata (epiphysic stippling), shortening of long bones, ichthyosis and hyperkeratosis. Autosomal recessive Greenberg skeletal dysplasia (MIM 215140), also known as HEM skeletal dysplasia, is a rare severe cholesterol biosynthesis defect that leads to early in utero lethality. The defect is due to mutations in the LBR gene causing a deficiency of the lamin B receptor (42), which functions as the 3β-hydroxysterol Δ4-reductase. Heterozygosity for a mutation in the lamin B receptor gene results in autosomal recessive Pelger-Huet anomaly (MIM 169400), a rare benign autosomal dominant disorder of leukocyte development characterized by hypolobulated nuclei and abnormal chromatin structure in granulocytes of heterozygous individuals (43).
Other disorders affect the biosynthesis of heme A and ubiquinone-10 and cause respiratory chain deficiencies (44;45). Mevalonate kinase deficiency (MKD) is the only disorder that affects the biosynthesis of all isoprenoids since mevalonate kinase (MK) functions in the pre-squalene part of the pathway. Because MKD is the subject of this thesis, it will be discussed in more detail in the following paragraph.

**MKD**

Originally, two distinct syndromes were defined to be caused by a deficiency of MK; classic mevalonic aciduria (MA, MIM 251170) (46) and the hyperimmunoglobulinemia D and periodic fever syndrome (HIDS, MIM 260920) (47). After the discovery that both disorders are caused by a deficiency of the same enzyme (48;49), due to mutations in the encoding MVK gene (MIM 251170), they are now recognized as the severe and mild ends of the MKD spectrum (50). Patients with the relatively mild HIDS phenotype suffer from recurrent episodes of fever, which often begin in the first year of life and occur every 3 to 6 weeks. The duration of those episodes varies between 2 to 7 days, most commonly 3 to 4 days. Fever rises abruptly, often above 40 °C, and the temperature then gradually returns to normal. The attacks can be precipitated by infections, minor trauma, childhood immunizations or physical and emotional stress, but usually occur with no clear precipitating event (51). The fever episodes are accompanied by malaise, headache, chills, sore throat, nasal congestion, arthralgia, arthritis, abdominal pain, diarrhea, vomiting, skin rash, hepatoplenomegaly and lymphadenopathy (51;52).

Patients with the severe MA phenotype experience similar fever episodes and present with additional symptoms such as developmental delay, dysmorphic features, cataracts, psychomotor retardation, hypotonia, myopathy and ataxia (53). Several severely affected patients died during a febrile crisis.

The majority of currently identified MKD patients with the HIDS presentation originate from the Netherlands. This may be a result of a heightened awareness of the disorder in the Netherlands and the inclusion of a specific laboratory test for IgD levels (one of the diagnostic hallmarks of HIDS) in Dutch patients with fever but can also partly be explained by a founder effect (54). In the Netherlands the carrier frequency of the most common HIDS mutation is estimated to be 1:350.

**IgD**

At first, all HIDS patients reported had constitutively raised serum IgD levels, usually accompanied with high levels of IgA (subclass IgA1). HIDS derived its name from this observation in 1984 (47). More recently, however, elevated levels of IgD and IgA have also been reported in MA (55,56) and some patients with other autoinflammatory diseases such as FMF (57), and TRAPS (58,59). Moreover, MKD patients with the HIDS phenotype, deficient MK activity and MVK mutations have been reported, who have normal IgD levels (49,60,61). These findings and the fact that no correlation between IgD concentration and disease severity is observed (47), make it unlikely that IgD plays a major role in the pathogenesis of MKD. Although IgD levels can be used as diagnostic hallmark, it remains necessary to perform MK enzyme activity measurements and/or mutation analysis of the MVK gene to establish the diagnosis.
Blood
When MKD patients experience a fever attack, a acute phase response is observed including leukocytosis, granulocytosis, increased erythrocyte sedimentation rates (ESR), high levels of serum amyloid A (SAA), C-reactive protein (CRP), fibrinogen, soluble type II phospholipase A2 and α1-glycoprotein (AGP) (51). The concentration of AGP is increased during attacks as well as between attacks, indicating a persistent state of inflammation (62). Activation of the cytokine network also occurs during the febrile episodes (63).

Cytokines
During febrile attacks, serum IFN-γ and IL-6 levels have been reported to rise sharply and TNF-α concentration increases until high normal. IL-1α and β levels are not elevated in serum of MKD patients during febrile attacks (63). The anti-inflammatory cytokines IL-1ra and soluble TNFR p55/p75 increase during attacks whereas IL-10 levels remained normal. The fact that no or only minor elevations of the proinflammatory cytokines TNF-α and IL-1β could be detected during attacks, does not exclude a role of these cytokines in the onset of attacks. Due to the delay between the onset of an attack and sampling, the possible rise in TNF-α and IL-1β levels could have been disappeared by the time of sampling.

In whole blood culture of blood obtained from MKD patients between attacks, IL-1β, IL-1ra and IL-6 levels did not change, but TNF-α concentrations are elevated. When blood was obtained during attacks, IL-1β and IL-6 levels remained unchanged but IL-1ra concentrations were increased. After stimulation with LPS, IL-1β was elevated in the whole blood cultures when blood was drawn during an attack. Between attacks, unstimulated isolated peripheral mononuclear cells (PBMCs) from HIDS patients produce significantly more IL-1β, IL-6 and TNF-α compared to control PBMCs and these cytokines rise further upon stimulation with LPS (64), suggesting in vivo priming of the monocytes/macrophages.

Biochemistry
In patients with the HIDS presentation a residual MK activity of up to 10% can be measured in cultured skin fibroblasts and PBMCs when compared to healthy control subjects, while in the severe MA presentation the MK activity is below detection levels. This difference in residual enzyme activity is also reflected in the plasma levels of the accumulating MK substrate, mevalonate (or mevalonic acid), which are moderately elevated in HIDS patients (ranging from 0.8-4.8 µmol/L) but can be massive in MA patients (up to a maximum of 500 µmol/L). In healthy control subjects these levels are typically below 0.03 µmol/L. Also the excretion of mevalonate in urine differs markedly between the two phenotypes. MA is characterized by a massive and constitutive excretion (1-56 mol/mol creatinine). In HIDS the excretion is moderate (0.005-0.040 mol/mol creatinine) and detectable more readily during febrile attacks (49). Excretion in HIDS patients is at least 5-fold higher than control levels between fever episodes and increases up to 100- to 500 fold during febrile attacks (65). In control subjects the excretion of mevalonate in urine is usually less than 0.001 mol/mol creatinine. Despite this marked difference in residual MK activity and accumulating levels of mevalonic acid, patients with the two presentations experience similar inflammatory episodes (53;66) suggesting that mevalonate itself has no effect
on the inflammatory response. In fact, increased intracellular mevalonate levels appear to be necessary to maintain the flux through (impaired) isoprenoid biosynthesis pathway. MA and HIDS cells compensate for the reduced MK activity by elevating their intracellular mevalonate levels through increasing HMG-CoA reductase activity (67). The increased HMG-CoA reductase activity was downregulated when MKD cells were incubated with the isoprenoid precursors farnesol (FOH), geranylgeraniol (GGOH) or mevalonate. Failure of this compensatory mechanism might lead to inflammation as suggested by the severe inflammatory crises provoked by lovastatin treatment of two MA patients in an attempt to lower the mevalonic acid levels in these patients (53). Under normal conditions, the elevated mevalonate levels and increased HMG-CoA reductase activity compensate for the MK deficiency and the flux through the isoprenoid pathway in MKD patients will be sufficiently high for most cellular processes requiring isoprenoids (68). However, any small increase in body temperature will result in a rapid decrease in residual MK activity, due to the deleterious, temperature-sensitive effect on MK protein maturation and stability of most mutations found in these patients (see below, (50;68)), which will lead to a rather instant disturbance of the flux through the pathway and, as a consequence, a temporary shortage of end products followed by inflammation and fever (68). This could provide an explanation for the episodic nature of the fever episodes in MKD.

The molecular background of MKD
Since the discovery of the molecular defect underlying HIDS (48;49) and MA (69) and the subsequent elucidation of the genomic structure of the MVK gene at chromosome 12q24 (70), many disease-causing mutations have been identified both at the cDNA and the genomic level. These include mostly missense mutations but also nonsense mutations, deletions, insertions, splicing defects and a complex mutation comprising a combination of a deletion and an insertion. In addition to these pathogenic mutations, several polymorphisms have been identified in the MVK gene (chapter 2).

The most common mutation in MKD is the c.1129G>A transition, which changes the valine at position 377 into an isoleucine (V377I). This mutation is exclusively associated with the HIDS phenotype and in fact is found in compound heterozygous state in the vast majority of patients with the HIDS phenotype. The A148T, N205D, P167L and T209A mutations also result in the HIDS phenotype and residual MK activities in MKD patients (chapter 2, (48;49;53;70;71)).

The disease-causing nature of the identified mutations was concluded from the fact that they all were found in patients clinically suspected to suffer from MK deficiency, whereas none of the mutations were found in control chromosomes. Moreover, most mutations were associated with marked deficient MK activities in cells of the patients. Several of the mutations have been expressed previously in Escherichia coli and were confirmed to have a deleterious effect on MK activity (49;72).

Most of the mutations in MKD affect stability and/ or folding of MK protein rather than affecting the catalytic properties of the enzyme. Mutations found in patients with the MA phenotype, however, have a more deleterious effect on MK protein folding and/or a direct effect on catalytic activity of the enzyme (chapter 2). Indeed, this latter has been demonstrated for the A334T mutation, which is located in a region of the MK protein involved in the binding of its substrate mevalonate. As a consequence of
Chapter 1

the A334T change, the Km for mevalonate has increased 30-fold (73), which means
that the enzyme is still catalytically active but requires far more mevalonate than
normally used in the enzyme activity assay to be measured accurately.

For MKD we found a rather good genotype-phenotype correlation. This is true for
both the clinical phenotype and the biochemical phenotype, in particular the residual
MK enzyme activity in cells from patients. Indeed, the measurement of MK activity in
patient cells appears more informative than predicting the effect of a certain mutation
on the basis of the crystal structure of MK (74), since, based on this structure, the
common V377I mutation was postulated not to have any significant effect on the
structure and activity of MK (75).

Treatment
There are currently no established therapies for MKD. Corticosteroids, colchicines,
intravenous immune globulin or cyclosporine alleviate symptoms in some patients
but none of these treatments is effective in the majority of patients (51). Moreover, the
long-term use of corticosteroids in childhood has too many adverse side effects to
justify their use as maintenance treatment to prevent attacks.

Simon et al. reported the outcome of simvastatin treatment of 6 patients with the
HIDS phenotype, which led to shorter periods of fever in most patients. However,
lovastatin treatment of two MA patients provoked severe inflammatory crises (53).
A pilot of etanercept (a recombinant p75 TNF receptor: Fc fusion protein) showed
substantial symptomatic improvement in two children with HIDS (61), but recently a
third patient was reported who did not respond to etanercept (76). In a clinical trial,
thalidomide (an inhibitor of TNF-α production) did not show an effect on disease
activity in HIDS patients (77).

More recently, treatment with the recombinant form of IL-1 receptor antagonist (IL-
1Ra) anakinra was shown to be effective in relieving the symptoms in MKD patients
(78-81).

Other autoinflammatory diseases

MKD is one of the hereditary periodic fever syndromes, a group of disorders
characterized by noninfectious recurrent inflammatory episodes with fever,
accompanied by abdominal pain, diarrhea, skin rash or arthralgia. The hereditary
periodic fevers include besides MKD, familial Mediterranean fever (FMF), tumor
genesis factor (TNF) receptor associated periodic syndrome (TRAPS), familial cold
autoinflammatory syndrome (FCAS), Muckle Wells Syndrome (MWS) and neonatal-
onset multisystem inflammatory disease (OMID)/ chronic infantile neurologic
cutaneous and arthropathy (CINCA) syndrome. They differ from autoimmune
diseases in lacking high titer autoantibodies or antigen specific T cells and are
therefore also called autoinflammatory diseases (82).

FMF
This autosomal recessive disorder is the most prevalent autoinflammatory disease,
originates from the Mediterranean basin and is most common amongst people from
Arab, Turkish, Armenian and Sephardic Jewish ancestry. FMF is characterized by
relatively short episodes of high fever accompanied by arthritis, erythematos rash,
pleuritis and severe abdominal pain due to serositis. Amyloidosis is a common complication of FMF, which leads, if left untreated, to renal failure (83). Treatment with daily oral colchicine prevents both the attacks (in most patients) and amyloidosis (84).

FMF is caused by mutations in the MEFL (MEditerranean FeVer) gene (85;86) encoding a protein called pyrin (also known as marenostrin). The pyrin domain (PYD) of this protein is a member of the death domain super fold family (87;88) and has homology with death domain (DD), death effector domain (DED) and caspase recruitment domain (CARD) subfamilies. All these domains participate in homotypic protein-protein interactions and are found in several proteins involved in the regulation of inflammation and apoptosis. Pyrin binds specifically to other proteins containing a pyrin domain including ASC (abbreviation for apoptosis associated speck-like protein containing a CARD). ASC has an N-terminal PYD and its C-terminal end encodes a CARD. The interaction of pyrin and ASC influences the activation of IL-1β, although the role of pyrin in the IL-1β pathway remains controversial. One hypothesis claims an inhibitory effect of pyrin on IL-1β activation. By binding to ASC and (pro-)caspase-1, pyrin would prevent them from being incorporated into the cryopyrin inflammasome, a complex that can activate IL-1β (89;90). This binding would be defective/compromised when pyrin is mutated, which then would lead to activation of IL-1β, causing the inflammatory phenotype in FMF.

In contrast, Yu et al. postulated a proinflammatory effect of pyrin (91). In their hypothesis, pyrin assemble an inflammasome complex with ASC and caspase-1 leading to ASC oligomerization, caspase-1 activation and IL-1β processing. The assembly is supposed to occur after the binding of pathogens to the C-terminal domain of pyrin (91). FMF-associated mutations would increase the binding of pathogens which then leads to increased inflammation in FMF.

In addition to these two hypothesis, there are also reports that claim that pyrin plays a role in regulating transcription factor NF-κB and apoptosis, however, the reported effects vary from inhibition to activation to no effect at all (91-94).

Cryopyrinopathies

FCAS, MWS and NOMID/ CINCA are three clinically distinct, dominantly inherited syndromes (‘the cryopyrinopathies’), which, however, are caused by mutations in the same gene, CIAS1, which encodes the protein cryopyrin (also referred to as NALP3, PYPAF1 or CATERPILLER 1.1) (95-97). FCAS is at the mild end of the clinical spectrum and is characterized by recurrent fever episodes with urticaria-like rash and polyarthralgia (98;99). Typically, these episodes are precipitated by exposure to the cold and last about 12-24 hours. In MWS the same rash occurs during the fever episodes as well as arthralgia, malaise, limb pain, and at times, abdominal pain and conjunctivitis (100). The duration of attacks is 2-3 days. Patients with MWS may also develop sensorineural hearing loss and/or systemic amyloid A (AA) amyloidosis. In the most severe cryopyrinopathy, NOMID/ CINCA, patients suffer from arthropathy, chronic aseptic meningitis, intellectual impairment and loss of vision in addition of the symptoms described for MWS patients (101). Eosinophilia or coagulopathy also occurs in some NOMID/ CINCA patients. Symptoms are continuously present but
fluctuate in severity. Overlapping forms of these clinical symptoms can be found between en within families (95).

Cryopyrin contains a pyrin domain (PYD), which interacts with ASC via PYD-PYD interactions. Together with caspase-1 and Cardinal (a CARD-containing protein), cryopyrin and ASC form the macromolecule complex called the cryopyrin inflammasome (102;103). This complex mediates caspase-1 activity, which is required to produce active IL-1β. Mutated cryopyrin activates the inflammasome, consequently leading to excessive amounts of mature IL-1β. Treatment of cryopyrinopathies therefore focusses on the reduction of IL-1β effect by IL-1 receptor antagonist anakinra. This treatment appeared to be successful in all three clinical syndromes (104-106).

TRAPS
TRAPS is an autosomal dominant disorder resulting from mutations in TNFSF1A gene, which encodes the 55-kDa receptor for TNF or type 1 TNF receptor (TNFR1)(107). This receptor is the major receptor for TNF-α induced signalling (108). TRAPS patients suffer their first attacks during childhood or adolescence. Fever episodes are accompanied by abdominal pain, pleurisy, peri orbital edema, arthralgia, arthritis and scrotal pain. The duration of attacks varies between 1 to 2 days, weeks or may even be continuous with fluctuating severity.

Activation of TNFR1 causes cleavage and shedding of its extracellular part, the soluble TNFR1, which can act as a TNF-α inhibitor by binding free TNF-α. Originally, studies indicated that mutations in the TNFSF1A gene lead to decreased shedding of TNFR1 (107). This could result in increased TNF-α signalling, which could explain the autoinflammatory phenotype of TRAPS patients. However, it can not explain the pathophysiology of all TRAPS patients since not all TNFR mutations result in defective shedding of p55TNFR (82;109-112).

Recent studies have indicated that some TNFR1 mutants are inefficiently expressed on the cell surface (111;113). This suggests that mutant TNFR1 proteins are misfolded and retain in the ER. These mutant TNFR proteins can form aggregates leading to TNF-independent signalling (114;115) or can result in an ER stress response which can lead to NF-κB activation and the induction of proinflammatory cytokines (114).

Treatment of TRAPS concentrates on inhibition of TNF by etanercept, a recombinant fusion protein of p75, the TNF receptor and the immunoglobulin fragment Fc (82) or infliximab, a humanized antiTNF antibody (116). In some TRAPS patients anti-TNF agent are rather ineffective which can be explained by TNF independent signalling due to abnormally retained TRAPS mutant TNFR1. Therapies directed at inhibiting expression, promoting degradation, or aiding correct folding of the mutant receptors may be additional targets for therapy in TRAPS. Also blocking downstream proinflammatory cytokines such as IL-1β might prove to be beneficial (117).

IL-1β
IL-1β is initially synthesized as an inactive 31 kDa precursor molecule (proIL-1β), and has to be proteolytically processed to become the active mature 17 kDa form (118;119). The synthesis, processing and release of mature IL-1β are tightly regulated events (120) and multiple signaling pathways are involved in the transcriptional
upregulation of proIL-1β, including pathways triggered by IL-1β itself, by other inflammatory cytokines such as TNF, or by various Toll-like receptor (TLR) ligands such as lipopolysaccharide (LPS) (121). The conversion of proIL-1β into the active mature 17 kDa IL-1β form requires the cleaving by the IL-1β converting enzyme (ICE, also known as caspase-1).

Caspase-1 is initially synthesized as an inactive 45 kDa precursor molecule (122). Procaspase-1 has to be converted into its active form through proteolysis at aspartic residues. The active form is composed of a 10 kDa and a 20 kDa subunit forming a heterodimer (122-124). A model for the activation of procaspase-1 is the so-called inflammasome (103). This complex comprises multiple monomers of caspase-1, caspase-5, ASC and NALP-1 (Nacht, LRR and Pyrin-domain containing protein 1). ASC can bind to caspase-1 via a CARD-CARD interaction (103;125-127), which brings caspase-1 and caspase-5 in close proximity leading to their cross-activation. Other members of the NALP family like NALP3/ cryopyrin are also able to form inflammasomes that can activate caspase-1 (102;128). Active caspase-1 converts proIL-1β into mature IL-1β, which is then rapidly secreted. Processing and release are closely linked. Activation of the nucleotide P2X7 receptor triggers the efflux of potassium ions out of the cell, which leads to the secretion of mature IL-1β by lysosomes (129). In support of the role of P2X7, overexpression of the receptor increases the secretion of IL-1β, and its absence prevents the secretion of IL-1β. The efflux of potassium ions triggers an influx of calcium ions, which in turn activates phospholipases. It appears that calcium-independent phospholipase A2 is required for caspase-1-processing in the specialized lysosomes, whereas phosphatidylcholine-specific phospholipase C is required for lysosomal exocytosis and release (129).

IL-1β is an important pro-inflammatory and pyrogenic cytokine which functions in different cell types. It stimulates the production of prostaglandins and nitric oxide, both of which are highly inflammatory. In addition, interleukin-1 induces the synthesis of chemokines, small proteins that facilitate the entry of neutrophils, macrophages, and lymphocytes into tissues, and the synthesis of other cytokines such as IL-6 (130).

IL-1β is part of the IL-1 family and is with IL-1α, IL-18 and IL-1Ra its best known member (131;132). IL-1α is also synthesized in a 31-kD proform and can be cleaved by calpain or extracellular proteases to a 17kD form. In contrast to IL-1β, IL-1α is already active in its proform. IL-1α is not secreted from cells, but remains intracellular or is membrane bound. It therefore functions by cell contact or after cell lysis. IL-1Ra binds to type I IL-1 receptors (IL-1RI) and blocks IL-1-dependent signal transduction. There is a natural balance between the proinflammatory activities of interleukin-1 and the blocking ability of interleukin-1–receptor antagonist. Like IL-1β, IL-18 is synthesized in a proform (24kD) and can be cleaved into its mature form (18kD) by caspase-1 (133;134). It also acts proinflammatory by inducing the synthesis of other proinflammatory cytokines and chemokines. In contrast to IL-1β, IL-18 is not an endogenous pyrogen (135). Recently, a novel member of the IL-1 family was described, IL-33. Similar as IL-1β and IL-18, IL-33 is produced in a proform and can be cleaved by caspase-1, at least in vitro (136). However, its biological activity differs from IL-1β and IL-18. IL-33 induces the production of Th2 associated cytokines as IL-4, IL-5 and IL-13, whereas IL-1β and IL-18 promote pro-inflammatory Th1 cytokines.
The common denominator of the various hereditary periodic fever syndromes seems to be the activation of the caspase-1 pathway resulting in IL-1β production. The key role of IL-1β is confirmed by the success with IL-1 receptor blockers in FMF, MKD, TRAPS and the cryopyrinopathies. Although IL-1ra is not successful in all MKD patients, IL-1β also seems to play an important role in the pathogenesis of MKD.

Aim and outline of this thesis

Overall aim of this PhD study was to get more insight in the mechanism by which a defect in the isoprenoid biosynthesis could lead to periodic inflammatory attacks and dysregulation of the immune system. More insight in the link between isoprenoid biosynthesis and immunodysregulation might lead to new options in treatment, not only in MKD, but potentially also in a number of other inflammatory disorders.

Chapter 1 provides an overview of the isoprenoid biosynthesis pathway, its defects with emphasis on MKD, the other autoinflammatory syndromes and IL-1β. In chapter 2 we describe all known mutations and the genotype-phenotype correlations in MKD. Chapter 3 describes the effect of MKD on the expression of genes and proteins involved in cytokine response. IL-1β is an important proinflammatory cytokine which seems to play a role in the pathogenesis of autoinflammatory diseases such as MKD. MKD leads to both an accumulation of mevalonate and a reduced production of isoprenoid endproducts. We previously described (chapter 6) that increased IL-1β secretion by MKD cells is due to a temporary shortage of certain isoprenylated proteins. Still, the question whether the accumulation of mevalonate or a shortage of isoprenoid endproducts is the cause of inflammation in MKD leads to discrepancies in literature. Using IL-1β as a read-out in chapter 4 we describe that a shortage of geranylgeranylated proteins rather than an excess of mevalonate is likely to cause increased IL-1β secretion by PBMCs of MKD patients. Which of the geranylgeranylated proteins (e.g. Rho, Rac, Cdc42) might play a role in the pathogenesis of MKD is still subject of investigation. In chapter 5 we studied the regulation of IL-1β maturation and secretion in impaired isoprenoid biosynthesis in the monocytic cell line THP-1. Our results suggest that inhibition of the isoprenoid biosynthesis pathway triggers caspase-1 mediated processing of pro-IL-1β and subsequent secretion of mIL-1β.

References

General introduction


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