Biochemical and genetic aspects of mevalonate kinase and its deficiency

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Chapter 9

Regulation of the mevalonate pathway in mevalonate kinase-deficient cell lines

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Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) are two inherited disorders both caused by depressed mevalonate kinase (MK) activity. MK functions in the isoprenoid biosynthesis pathway and is the first enzyme to follow the highly regulated HMG-CoA reductase (HMGR), which catalyzes the rate-limiting enzyme step. In cells from MA patients, HMGR enzyme activity is elevated continuously mainly due to a shortage of nonsterol isoprenoid endproducts. This was indicated by normal HMGR mRNA levels in MA fibroblasts and an efficient downregulation of HMGR enzyme activity when cells were cultured in the presence of the isoprenoid precursors farnesol, geranylgeraniol and mevalonate but not with cholesterol. Thus, it appears that elevated mevalonate levels allow cells to compensate for the deficiency in MK activity. Indeed, isoprenylation of proteins was normal in HIDS and MA fibroblasts, but had increased sensitivity toward inhibition of HMGR by simvastatin. This illustrates that MK deficient cells maintain their pathway flux by elevating intracellular mevalonate levels.

Introduction
Hyper-IgD and periodic fever syndrome (HIDS, MIM 260920) and mevalonic aciduria (MA, MIM 251170) are two autosomal recessive disorders both caused by a deficient activity of the enzyme mevalonate kinase (MK, E.C. 2.7.1.36) due to functional significant mutations in the encoding gene (MVK)[1-5]. MA is a severe and often fatal multi-systemic disease, characterized by psychomotor retardation, failure to thrive, hepatosplenomegaly, anemia and recurrent febrile episodes. HIDS is a relative benign condition, in which patients suffer, as in MA, from recurrent fever episodes associated with lymphadenopathy, arthralgia, gastrointestinal problems and skin rash.

MK enzyme activity in MA is usually below detection levels when measured in cultured skin fibroblasts of MA patients [2]. In HIDS, however, a residual MK activity varying between 1 and 7% of the control value can be measured both in fibroblasts and leukocytes from patients [4, 6]. As a result of the MK deficiency, excretion of mevalonic acid in urine occurs, which correlates with disease activity in both syndromes. The basal level of excreted mevalonic acid, however, is much higher in MA [2, 7].

MK is the first enzyme to follow the highly regulated 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMGR) in the isoprenoid biosynthesis pathway and converts mevalonate into 5-phosphomevalonate. This pathway provides cells with isoprenoids that are vital for diverse cellular processes. The main end-products include isoprenylated proteins, heme A, dolichol, ubiquinone-10, isopentenyl tRNAs and sterols. 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 HMGR) and its receptor-mediated uptake from plasma lipoproteins (LDL-receptor) [8]. This regulation is performed by a class of transcription factors called sterol regulatory element binding proteins (SREBPs) [9]. These are conditional positive transcription factors that enhance transcription when sterols are absent, but are not required for basal transcription when sterols are present [8]. HMGR, which performs the rate-limiting enzyme step in isoprenoid biosynthesis, is also subject to several post-transcriptional regulation mechanisms. These include translational efficiency of the HMGR mRNA and turnover of the HMGR protein [8]. The rate of translation of HMGR mRNA is dictated by the cell's demand for non-sterol isoprenoids, whereas the degradation rate of the HMGR protein is regulated by the
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Cell's demand for both sterol and non-sterol isoprenoids [8]. The sterols probably act via the membrane-spanning domain of HMGR, which is not necessary for catalytic activity, but has a so-called 'cholesterol sensing domain' [8]. Farnesol (FOH) has been implicated to be a non-sterol regulator of HMGR degradation [10-16], however this is still a matter of debate [17]. Combined, these regulatory mechanisms can induce a 200-fold increase in HMGR protein in response to statins [8]. Statins are potent competitive inhibitors of the HMGR and are widely used to treat atherosclerosis and familial hypercholesterolaemia. These drugs block the synthesis of mevalonate and, as a consequence, lower the endogenous synthesis of isoprenoids.

Although fibroblasts from MA patients have virtually no detectable MK enzyme activity, biosynthesis of cholesterol from radiolabeled precursors can be rather normal depending on the culture conditions [18, 19]. Thus, it appears that MA fibroblasts are able to compensate for their defect in MK and that the pathway flux may be near normal. This is due to increased activity of HMG-CoA reductase and the LDL receptor pathway [18, 19]. This increased activity of HMG-CoA reductase is unsuppressible by exogenous LDL cholesterol and is further upregulated under cholesterol-free culture conditions [18], suggesting that the high basal HMGR activity in MA is not due to a shortage of sterol end-products. We have extended these studies by studying the effect of nonsterol end-products on HMGR activity and determining the effect of HMGR inhibition on protein prenylation.

Material and methods

Materials

HMG-CoA, geraniol (GOH), FOH and geranylgeraniol (GGOH) were obtained from Sigma. Radiolabeled 14C-HMG-CoA was obtained from Amersham Pharmacia Biotech. When necessary the obtained batch was purified further by ethylacetate extraction. The antibody against Ras was obtained from Transduction Laboratories, the antibody against RhoA was obtained from Santa Cruz Biotechnology.

Cell culture

Fibroblast cell lines were cultured in Nutrient Mixture Ham's F-10 with L-glutamine and 25 mM HEPES (Gibco) supplemented with 10% fetal calf serum (FCS, Gibco) or 10% delipidated FCS (Roche) as indicated. For each experiment cells were seeded in a T75 culture flask (Costar) and grown until confluency. Two days prior to a particular treatment the cells received fresh culture medium. To this end, culture medium with the indicated compound was added to the cells and the incubation was continued for another 2 days. For enzyme and immunoblot analysis cells were harvested and washed twice with PBS after trypsinization, and either used directly or snap-frozen in liquid nitrogen and stored at -80°C until use.

Mevalonolactone, GOH, FOH and GGOH were dissolved in ethanol. Mevalonic acid was prepared by dissolving the mevalonolactone in a NaOH solution. A simvastatin stock solution was prepared by dissolving the prodrug in ethanol, followed by hydrolysis of the lactone by adding NaOH. After neutralization with 1 M HEPES pH7.4 and HCl the solution was sterilized by filtration through a 0.2 μm filter and stored in aliquots at -20°C.

All patients and patient cell lines had low MK enzyme activity in combination with mutations in the encoding MVK gene.
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Quantitative PCR
The relative expression levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and HMGR mRNAs were measured with the lightcycler system (Roche). To this end, total RNA (free of genomic DNA) was isolated with the SV RNA total isolation system (Promega) after which first strand cDNA was prepared as described by IJlst et al. [20]. The relative mRNA expression levels of HMGR and GAPDH were determined using a plasmid containing the corresponding gene as the standard. The GAPDH fragment was amplified using the following primer set: GAPDH Fw, 5'-ACC ACC ATG GAG AAG GCT GG-3', and GAPDH Rev, 5'-CTC AGT GTA GCA GAT GC-3'. The HMGR fragment was amplified using primers: HRED Fw2, 5'-TC A AA G GG T AC A GA G AA A GC A C-3', and HRED Rev2, 5'-TAT GCT CCC AGC CAT GGC AG-3'. In every sample the expression of GAPDH and HMGR was determined in duplicate.

HMGR enzyme assay
HMGR was measured essentially as described by Brown et al. [21] with some modifications. The fibroblast pellets were dissolved in HMGR assay buffer containing 100 mM KPi, 200 mM KCl, 5 mM EGTA, 5 mM EDTA, 10 mM DTT and 10 µg/ml leupeptin (pH 7.1). The cells were disrupted by sonication (twice at 8 W output, 40 J, at room temperature). Hundred microliter of the resulting homogenate was preincubated for 10 min at 37 °C with 60 µl of cofactor-mix containing 66.7 mM glucose-6-phosphate, 10 mM NADPH, 16.7 mM EDTA and 25 U/ml glucose-6-phosphate dehydrogenase. The enzyme reactions were started with the addition of 1.7 nmol of [14C]-HMG-CoA and 5.6 nmol HMG-CoA in 40 µl H2O. After a 30 min incubation period at 37 °C, reactions were terminated by adding 50 µl of 1.2 N HCl. After 30 min, the product was extracted three times with 2 ml of ethylacetate. The extracts were evaporated to dryness and analyzed by silica thin layer chromatography using a solvent system toluene : acetone (1 : 1) dried with Na2SO4. The formed product was quantified by PhosphorImaging (Fuji FLA-3000) with the aid of the Aida software package using samples with known amounts of 14C-mevalonate. In every sample the activity HMGR was determined in duplicate.

Membrane and cytosol separation
Cell pellets were dissolved in hypotonic buffer containing 5 mM Tris.Cl pH7.0, 5 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 2 mM EGTA, 2 mM DTT and Complete protease inhibitor cocktail (Roche). The cells were lysed by sonication (twice at 8 W output, 40 J, with cooling between the pulse periods). The membranes were separated from the cytosolic fraction by a 30 minutes ultracentrifugation step in an airfuge (Beckman, 15 psi, 100,000g). The supernatant was designated as the cytosolic fraction. The pellet was dissolved in RIPA++ buffer containing 20 mM Tris.Cl pH8.0, 150 mM NaCl, 10 mM NaH2PO4, 5 mM EDTA, 10% glycerol, 1% NP-40, 1% Na-deoxycholic acid, 0.1% SDS, 1 mM DTT and Complete protease inhibitor cocktail and homogenized by sonication (once 7 W output, 40J). Both fractions were boiled in Laemmlı sample buffer and stored at ~20°C.

Immunoblot analysis
Equal amounts of protein (measured in the sonicated lysates prior to ultracentrifugation) were resolved on a 15% SDS-polyacrylamide gel and transferred onto nitrocellulose by semi-dry immunoblotting. As a control for equal transfer of protein, the blots were stained reversibly
with Ponceau S. Membranes were blocked using blocking buffer containing 5% non-fat dry milk and 1% bovine serum albumin in PBS with 0.1% Tween. Membranes were probed with either the Ras antibody (1:1000) or the RhoA antibody (1:1000) in blocking buffer which was diluted 10 times in PBS with 0.1% Tween. Detection of the antigen-antibody complexes was performed with horseradish peroxidase conjugated secondary antibody (DAKO) and the enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech).

Results

_Elevated HMG-CoA reductase activity is suppressed by FOH and GGOH_

As previously reported by Gibson et al. [18], we observed an elevation of HMGR activity in cultured skin fibroblasts of MA patients, while in HIDS fibroblasts, HMGR activity was within the normal range [22]. Gibson et al. also reported [18] that the elevated HMGR enzyme activity in MA fibroblasts could not be suppressed by exogenous LDL cholesterol and was further upregulated in the absence of LDL cholesterol in the FCS [18]. We also observed this additional upregulation when cells were cultured under lipid-free conditions (data not shown). These findings suggest that the elevated activity is due to one of the post-transcriptional non-sterol dependent regulatory mechanisms for HMGR enzyme activity. Analysis of HMGR mRNA by quantitative PCR and determination of the activity of HMGR indeed revealed that the elevated activity in the MA fibroblasts was not due to a higher transcription rate of the HMGR mRNA (Table 1). Therefore, we tested whether the HMGR activity in MA fibroblasts could be suppressed by nonsterol isoprenoids. To this end, MA and control fibroblasts were cultured for two days with different concentrations of GOH, FOH or GGOH (figure 1). This treatment caused a dose-dependent decrease of the elevated HMGR enzyme activity in MA fibroblasts. GGOH was the most effective in suppressing HMGR enzyme, whereas FOH was more effective than GOH. GGOH also suppressed HMGR activity in control fibroblasts. This suppression of HMGR enzyme activity by nonsterol isoprenoids was fast with an almost maximal effect already after 2 hours (data not shown).

**Table 1.** Relative mRNA expression levels of HMGR and HMGR enzyme activity in control, HIDS and MA cultured skin fibroblasts.

<table>
<thead>
<tr>
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<th>Relative HMGR expression 500xHMGR/GAPDH</th>
<th>HMGR activity pmol/min.mg</th>
<th>Activity/expression</th>
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<tr>
<td>HIDS</td>
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<tr>
<td>MA</td>
<td>1.2±0.2</td>
<td>58±9</td>
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_Exogenous mevalonate suppresses HMGR activity in MA fibroblasts_

Since fibroblasts from MA patients are still capable of synthesizing cholesterol and other isoprenoids from radiolabeled precursors like acetate [18, 19], MK activity is not entirely deficient in these cells despite the undetectable levels in our MK enzyme assay. To test whether exogenous mevalonate is capable of normalizing HMGR enzyme activity in MA fibroblasts, these cells were cultured for 48 hours in the presence of mevalonolactone or sodium mevalonate (figure 2). This treatment caused a dose-dependent decrease in HMGR enzyme activity. Sodium mevalonate was more effective than mevalonolactone in suppressing HMGR enzyme activity.
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**Figure 1.** HMG R specific activity in control (C) and MA (M1, M2 and M3) cell lines after 48 hours of culturing in the presence or absence of the isoprenols, GOH, FOH and GGOH. The error bars indicate 1 SD.

**Figure 2.** HMG R specific activity in control (C) and MA (M1 and M2) cell lines after 48 hours of culturing in the presence or absence of mevalonate or mevalonolactone at different concentrations. The error bars indicate 1 SD.
HIDS and MA skin fibroblasts have increased sensitivity for simvastatin

From our results we hypothesize that MA and HIDS fibroblasts compensate for their depressed MK activity by raising intracellular mevalonate levels. In order to test this hypothesis we determined the sensitivity of a control, a HIDS and a MA fibroblast cell line to inhibition of HMGR by simvastatin. This was done by culturing these cell lines in the presence of different concentrations simvastatin for 48 hours. After incubation, the cells were fractionated into a membrane and a cytosolic fraction which were subjected to immunoblotting with antibodies against two prenylated proteins (figure 3). Most prenylated proteins function in the membrane and need farnesyl or geranylgeranyl moieties to become associated with the membrane. As a consequence of normal protein turnover, inhibition of HMGR by simvastatin will lead to an increase in unprenylated (and non-functional) proteins in the cytosolic fraction and a decrease in prenylated proteins in the membrane fraction [23, 24]. We used antibodies to Ras, which is a farnesylated protein, and RhoA, which is a geranylgeranylated protein. All fibroblast cell lines cultured in the absence of simvastatin had similar levels of Ras and RhoA protein in the membrane fraction, indicating that MA and HIDS fibroblasts are capable of synthesizing prenylated proteins as efficiently as control fibroblasts (figure 3). Similar results were obtained in cultured lymphoblasts of MA and HIDS patients (data not shown). It appears that in confluent grown fibroblasts the majority of the RhoA protein is localized in the cytosol fraction, whereas the majority of Ras is localized in the membrane fraction.

Depletion of cellular mevalonate levels induced by simvastatin treatment of the cells showed a marked difference. First, in all cell lines, total RhoA protein levels are upregulated in response to simvastatin treatment, which appears a compensatory response to the lowered levels of functional RhoA in the membrane. In addition, the MA cell line had more RhoA in the cytosolic fraction than the control cell line. Moreover, in MA and HIDS fibroblasts, both Ras (figure 3a) and RhoA (figure 3b) appear in the cytosolic fraction at lower concentrations of simvastatin than controls. In fibroblasts from an MA patient an effect was already visible at a simvastatin concentration as low as 8 nM. Also fibroblasts from a HIDS patient were more sensitive to simvastatin than a control cell line. Although both the control and the HIDS cell line start to accumulate RhoA in the cytosol at 40 nM simvastatin, this process is faster in the HIDS cell line. In addition, quantification of the intensity revealed that this process was already maximal at 200 nM in the HIDS cell line, whereas in the control cell line it was only half-maximal at this concentration (data not shown). Thus, the extent of the MK deficiency reflects the sensitivity to simvastatin, with the cells harboring the lowest MK activity being the most sensitive.

In order to study the effect of an increased pathway flux on the sensitivity to simvastatin, MA, HIDS and control fibroblasts were cultured in lipid-free FCS. The sensitivity to inhibition of HMGR by simvastatin decreased in all cell lines, which most probably reflects the upregulation of HMGR enzyme activity (figure 3b). In MA and HIDS fibroblasts, however, RhoA appears in the cytosolic fraction at lower concentrations of simvastatin than controls. Without addition of simvastatin, lipid-free FCS did not induce a difference in isoprenylation between MA, HIDS and control fibroblast cell lines.
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**A**

Simvastatin Cytosol Membranes

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**B**

FCS Lipid-free FCS Simvastatin Lipid-free FCS Simvastatin Simvastatin

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**Figure 3.** A. Immunoblot analysis of Ras in cytosol and membrane fractions of fibroblast lysates (15 µg of protein/lane). The control, HIDS and MA cell line were cultured in the presence of different concentrations of simvastatin. The concentrations are indicated in the figure. **B.** Immunoblot analysis of RhoA in cytosol and membrane fractions of fibroblast lysates (12.5 µg of protein/lane and 25 µg of protein/lane, respectively). The control, HIDS and MA cell line were cultured in the presence of different concentrations of simvastatin in medium containing FCS and medium containing lipid-free FCS. The concentrations are indicated in the figure. Shown are the results from one representative experiment from 3 independently performed experiments.

**FOH and GGOH can rescue deficient isoprenylation in HIDS and MA**

FOH and GGOH can be utilized by cells for isoprenoid biosynthesis when added to the culture medium [25-27]. We tested whether fibroblasts from an MA patient were able to use FOH and GGOH for rescuing protein prenylation when HMGR was inhibited by simvastatin. FOH rescued farnesylation of Ras (figure 4a) as judged from the decreased level of this protein in the cytosol, whereas GGOH rescued geranylgeranylation of RhoA (figure 4b) as judged from the increased level of this protein in the membrane fraction. A combination of both compounds rescued prenylation of both proteins. Also the addition of 1 mM mevalonate...
to the medium rescued prenylation of RhoA, again indicating that MA fibroblasts can use mevalonate when concentrations are high enough (figure 4b). In line with the above suggested correlation between membrane-bound RhoA versus total RhoA levels, we observed that FOH, GGOH and mevalonate decreased the level of RhoA in the cytosol (figure 4b).

**Figure 4.** A. Immunoblot analysis of Ras in cytosol and membrane fractions of fibroblast lysates of a MA patient (10 µg of protein/lane and 20 µg of protein/lane, respectively) showing the effect of 20 µM FOH and 20 µM GGOH alone or in combination on the treatment with simvastatin. B. Immunoblot analysis of RhoA in cytosol and membrane fractions of fibroblast lysates of a MA patient (10 µg of protein/lane and 20 µg of protein/lane, respectively) showing the effect of 20 µM FOH and 20 µM GGOH alone or in combination on the treatment with 40 nM simvastatin (lane 2-5) and the effect of 1 mM mevalonate on the treatment with 100 nM simvastatin (lane 6 and 7).

**Discussion**

MA and HIDS are syndromes both caused by a deficiency of MK enzyme activity. Despite this deficiency, cholesterol biosynthesis in fibroblasts derived from MA patients can be normal depending on the culture condition [18, 19]. Thus, it appears that MA fibroblasts are able to compensate for their defect in MK. This is possible because they have increased activity of HMGR and the LDL receptor pathway [18, 19]. HMGR, which catalyzes the enzyme step preceding the one catalyzed by MK and 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 [8]. The increased activity of HMGR in MA fibroblasts was unsuppressible by exogenous LDL cholesterol and was further upregulated under cholesterol-free culture conditions [18], suggesting that the high basal HMGR activity
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in MA is not due to a shortage of sterol end-products. Apparently, the lipoproteins present in the FCS supply the cells with enough cholesterol, preventing SREBP activation. In accordance, we found that HMGCR mRNA levels are normal in MA fibroblasts, indicating that the sterol-dependent SREBP pathway (transcriptional) is not activated. The increased HMGCR activity, however, was downregulated when the medium of MA cells was supplied with FOH, GGOH, or extra mevalonate. This suggests that one of the non-sterol dependent regulatory mechanisms causes the increase in activity. These mechanisms are post-transcriptional and involve higher mRNA translation efficiency and decreased protein turnover. FOH has been implicated to be a nonsterol regulator of HMGCR activity since it accelerates HMGCR protein turnover [10-16]. In our experiments, GGOH was more effective than FOH in downregulating HMGCR enzyme activity. Correll et al. [13] reported that FOH was more effective than GGOH in promoting HMGCR protein degradation, suggesting that the effect of GGOH may be related to mRNA translation efficiency. The difference in efficacy of mevalonate and mevalonolactone is probably a reflection of the absence of specific hydrolases (esterases) for mevalonolactone in fibroblasts and the different diffusion coefficients of both molecules for crossing the cellular membrane.

As already reported for cholesterol biosynthesis from radiolabeled precursors, we now report that under normal conditions protein isoprenylation in HIDS and MA fibroblasts is normal. In both cell lines, Ras, a farnesylated protein, and RhoA, a geranylgeranylated protein, were present in the membrane fraction as shown by cellular subfractionation followed by immunoblotting. Thus, it appears that MA and HIDS cells are able to compensate for reduced MK activity by elevating their intracellular mevalonate levels. This was also illustrated by the fact that addition of extra mevalonate to the medium downregulated HMGCR activity in MA fibroblasts. In this case, the elevated HMGCR activity as observed in MA fibroblasts serves mainly to compensate for the leakage of mevalonate (or mevalonolactone) out of the cell. Accordingly, MA fibroblasts are more sensitive to simvastatin than HIDS fibroblasts, whereas HIDS fibroblasts are more sensitive to HMGCR inhibition than control fibroblasts. This was demonstrated by the variable accumulation of unprenylated proteins in the cytosol after treatment of cells with different concentrations of statins.

Recently, it was reported that the protein levels of four small isoprenylated GTPases, including RhoA, increased in response to mevalonate depletion [28, 29]. We also observed that treatment of fibroblasts with simvastatin increased the RhoA protein level. In addition, the basal expression levels of RhoA in the cytosolic fraction of MA fibroblasts appears upregulated. This could indicate that beside elevated mevalonate levels, also higher RhoA protein levels are necessary for sustaining sufficient geranylgeranylation of RhoA. Therefore, it is necessary to develop sensitive methods to measure the intermediates of isoprenoid biosynthesis. This would enable more detailed studies towards the effect of MK deficiency on the concentrations of these intermediates. It may be the only proper way for measuring the effect of MK deficiency on the pathway flux.

The elevation of intracellular mevalonate concentrations may promote a normal pathway flux when the following three criteria are met: (1) MK is not saturated with substrate. When MK is saturated any elevation in mevalonate has no effect; (2) HMGCR is able to provide mevalonate levels that are high enough for MK to function at a normal rate. HMGCR has to compensate for the leakage of mevalonate out of the cell; (3) HMGCR is not subjected to noncompetitive product inhibition. This is not the case because HMGCR is insensitive to any form of product inhibition [30].
Thus, MA and HIDS fibroblasts are able to compensate largely for their deficiency in MK by elevation of intracellular mevalonate levels. This could imply a pathogenetic mechanism in which toxic accumulation of mevalonate would be the cause of the observed symptoms in HIDS and MA. Hoffmann et al., however, reported that a trial with lovastatin in 2 MA patients, used in order to lower mevalonate levels, resulted in severe clinical crises [2]. This clearly indicates that an excess of mevalonate itself is not the pathogenic factor in MA, but a shortage of one of the isoprenoid end-products. In fact, it illustrates the importance of maintaining elevated mevalonate levels.

We have additional evidence that the pathogenesis of HIDS and MA may be related to a shortage of some isoprenoid end-product. The common V377I mutation identified in most HIDS patients affects MK activity in a temperature sensitive manner, which means that when cultured at temperatures lower than 37°C the MK enzyme activity increases, whereas when cultured at higher temperatures the activity decreases. A similar phenomenon was observed in peripheral blood mononuclear cells (PBMCs) of HIDS patients. The PBMCs had significantly lower MK enzyme activities during fever than between fever episodes. In addition, the wild-type MK enzyme activity also displayed this temperature sensitivity, although to a lesser degree [22]. This renders it plausible that in MA fibroblasts MK also shows temperature sensitivity, at least to some degree. Thus, a small additional decrease in MK activity in MA and HIDS cells as can be caused by an increase in temperature may have far reaching consequences. In HIDS cells, the steady-state levels of MK protein are very low and the enzyme approaches saturation. Consequently, the relative increase in intracellular mevalonate level must be much higher than in control cells in order to keep the pathway flux normal, which leads to a temporary decrease in pathway flux and the production of isoprenoids. This is reflected by a compensatory increase in HMG R activity indicating that MK becomes progressively rate-limiting [22]. 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 anti-inflammatory isoprenoids, followed by inflammation and fever. In MA cells, MK is likely to be saturated with its substrate already, thus an additional increase in mevalonate concentration would have little or no effect. This may be the case when a MVK mutation results in reduced MK protein levels. Additionally, HMG R 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 [31]. This suggests that the pathogenesis of HIDS and MA does not involve a continuous shortage of endproducts, but a temporary reduction in pathway flux, which may provide an explanation for the discontinuous disease-course of both disorders.

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