ONCOIMMUNOLOGY
2017, VOL. 6, NO. 10, e1342917 (10 pages)
https://doi.org/10.1080/2162402X.2017.1342917

Taylor & Francis
Taylor & Francis Group

REVIEW

Mevalonate metabolism governs cancer immune surveillance

Georg Gruenbacher and Martin Thurnher

Immunotherapy Research Unit, Department of Urology, Medical University of Innsbruck, Innsbruck, Austria

ABSTRACT

The metabolic reprogramming that drives immunity engages the mevalonate pathway for cholesterol biosynthesis and protein prenylation. The importance of tight regulation of this metabolic route is reflected by the fact that too low activity impairs cellular function and survival, whereas hyperactivity can lead to malignant transformation. Here, we first address how mevalonate metabolism drives immunity and then highlight ways of the immune system to respond to both, limited and uncontrolled flux through the mevalonate pathway. Immune responses elicited by mevalonate pathway dysregulation may be harnessed to increase the clinical efficacy of current cancer therapy regimens.

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACAT-1, acetyl-CoA acetyltransferase 1; ATP, adenosine triphosphate; ACLY, ATP citrate lyase; BTN, butyrophilin; CRM, caloric restriction mimetic; DMAPP, dimethylallyl diphosphate; FA, fatty acid; FPP, farnesyl diphosphate; GM-CSF, granulocyte/macrophage-colony-stimulating factor; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; GTP, guanosine triphosphate; HC, hydroxycitrate; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IPP, isopentenyl diphosphate; mTOR, mechanistic target of rapamycin; N-BP, nitrogen-containing bisphosphonate; OXPHOS, oxidative phosphorylation; PI3K, phosphoinositide 3-kinase; ROCK, Rho-associated protein kinase; SCAP, SREBP cleavage-activating protein; SREBP, sterol regulatory element-binding protein; TCA, tricarboxylic acid; TCR, T cell receptor; Th, T helper; TLR, toll-like receptor

ARTICLE HISTORY
Received 22 March 2017
Revised 8 June 2017
Accepted 9 June 2017

KEYWORDS
ATP citrate lyase; cancer; cholesterol; dendritic cells; flux; HMG-CoA reductase; immunometabolism; macrophages; mevalonate; protein prenylation; T lymphocytes

Although important observations made by Otto Warburg in cancer cells date back several decades, only now the general requirement of metabolic reprogramming for the shift of a cell from quiescence to an activated state, has become obvious. Also in immune cells, dramatic metabolic changes are required to effectively perform the diverse cellular functions that constitute a productive immune response against invading pathogens and tumor cells. These changes also include the enhanced engagement of the mevalonate pathway, best known for the biosynthesis of cholesterol. It has been the seminal work of Michael Brown and Joseph Goldstein, awarded by the Nobel Prize in Physiology or Medicine in 1985,[1] which represented the foundation for further intense investigation of mevalonate metabolism. Although immunometabolism has become a distinct category of immunological research in the past few years, mevalonate metabolism still receives little attention.[2,3] The purpose of this review is to provide a general overview of mevalonate metabolism in immune cells and to highlight ways of the immune system to respond to mevalonate pathway dysregulation.

Mevalonate metabolism

T cells

The rapid increase in glycolysis is a fundamental feature of T cell activation.[4-6] The increase in aerobic glycolysis ensures the availability of biosynthetic building blocks required for cell growth, proliferation, differentiation and effector function. In metabolic reprogramming, the tricarboxylic acid (TCA) cycle, is a center of metabolic activity. To initiate lipogenic pathways, some of the mitochondrial citrate is exported to the cytosol and cleaved by ATP citrate lyase (ACLY) to generate cytosolic acetyl-CoA, which then serves not only as a key metabolite for fatty acid (FA) biosynthesis but also directly fuels the growth-promoting mevalonate pathway (Fig. 1).[7] In a recent study, a nuclear phosphoproteomic screen revealed that ACLY is a key phosphoprotein effector of interleukin (IL)-2-mediated T-cell responses.[8] ACLY becomes phosphorylated on serine 455 in T lymphocytes upon IL-2-driven activation.[8] Conversely, depletion or inactivation of ACLY compromised IL-2-promoted T cell growth. ACLY has previously been shown to be a substrate of AKT,[9] which has several downstream effects including activation of the key metabolic sensor mTOR (mechanistic target of rapamycin). mTOR, which critically regulates lymphocyte metabolism, consists of 2 complexes, mTORC1 and mTORC2.[10] mTORC1 increases protein translation via phosphorylation of 4E-BP1 and p70S6 kinase[11] and controls the expression of numerous genes involved in mevalonate metabolism mainly through the sterol regulatory element-binding protein (SREBP) transcription factors (Fig. 2).[12, 13] The entire pathway of SREBP2 target genes has been shown to be induced after T cell receptor triggering,[14] emphasizing the importance of mevalonate metabolism for T cell activation.

CONTACT Martin Thurnher martin.thurnher@i-med.ac.at Immunotherapy Research Unit, Department of Urology, Medical University of Innsbruck, Innrain 66a, 6020 Innsbruck, Austria – Europe.

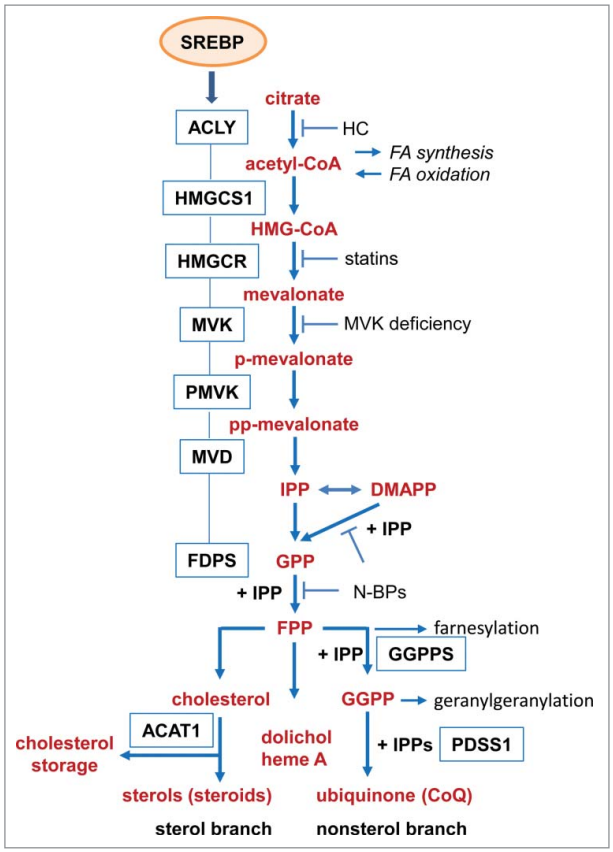

Figure 1. The SREBP-driven mevalonate pathway. The transcriptional activity of sterol regulatory element-binding proteins (SREBPs) governs expression of mevalonate pathway enzymes. ATP citrate lyase (ACLY, EC 2.3.3.8), which can be inhibited by hydroxycitrate (HC), generates cytosolic acetyl-coenzyme A (acetyl-CoA). 3-hydroxy-3-methylglutaryl CoA (HMG-CoA)-synthase (HMGCS1, EC 2.3.3.10) condenses 3 molcules of acetyl-CoA to form HMG-CoA. HMG-CoA reductase (HMGCR, EC 1.1.1.34), which can be inhibited by statins, in turn generates mevalonate (also known as mevalonic acid) in the first committed step of the pathway. Sequential phosphorylation reactions by mevalonate kinase (MVK, EC 2.7.1.36), which is mutated in MVK deficiency, and phosphomevalonate kinase (PMVK, EC 2.7.4.2), respectively, followed by a decarboxylation step catalyzed by mevalonate diphosphate decarboxylase (MVD, EC 4.1.1.33) produce isopentenyl diphosphate (IPP). Farnesyl diphosphate (FPP) synthase (FDPS, EC 2.5.1.10), which is the target of nitrogen-containing bisphosphonates (N-BPs) condenses IPP (C_5) and its isomer dimethylallyl diphosphate (DMAPP, C_5) to form geranyl diphosphate (GPP, C_{10}) and subsequently GPP with another IPP unit to generate FPP (C_{15}). FPP is the common precursor for cholesterol and steroids in the sterol branch as well as for products of the nonsterol branch including geranylgeranyl diphosphate (GPPP, C_{20}), dolichol, heme A and ubiquinone (coenzyme Q). GGPP is formed by GGPP synthase (GGPS, EC 2.5.1.29) through condensation of FPP with yet another IPP unit. In protein prenylation, FPP and GGPP, serve as prenyl group donors in posttranslational modifications of multiple Ras protein family members and G protein-coupled receptors. Farnesylation (using FPP) or geranylgeranylation (using GGPP) are required for membrane attachment and function of these proteins. Prenylated proteins constitute approximately 0.5% to 2% of proteins in mammalian cells. Prenyl-diphosphate synthase-1 (PDSS1, EC 2.5.1.91) catalyzes the elongation of GPP or FPP with several IPP moieties to form the polyisoprenoid chain of ubiquinone (coenzyme Q). ACAT1 catalyzes cholesterol esterification for storage purposes.

Two major branches of mevalonate metabolism (Fig. 1) have emerged as important regulators of T lymphocyte biology. The sterol branch for cholesterol biosynthesis critically regulates T cell cycle progression and effector function. Activated CD8 T cells therefore rapidly reprogram their metabolism through the actions of the SREBP and liver X receptor (LXR) transcription factors to ensure cholesterol availability by promoting cholesterol biosynthesis, while concomitantly decreasing cholesterol efflux.[14] Activated $CD8^+$ T cells can further increase plasma membrane levels of free cholesterol by preventing cholesterol esterification for storage.[15, 16] Specific inhibition of the cholesterol esterification enzyme ACAT1 (Fig. 2) improved immunological synapse formation and TCR signaling, resulting in enhanced production of cytokines, degranulation and proliferation of $CD8^+$ T cells. ACAT-1 might also be an attractive therapeutic target in tumor therapy (Fig. 3), since ACAT1 inhibition has already been shown to improve the function of antitumor $CD8^+$ T cells reactivated by immune checkpoint blockade to treat melanoma in mice.[16, 17]

The nonsterol branch for protein prenylation (Fig. 1) also determines multiple aspects of T cell function, including synapse formation, migration, proliferation and cytotoxic effector responses.[6] The prototype of small guanosine triphosphatases (GTPases) Ras is activated through prenylation in response to TCR stimulation and various cytokines. In protein prenylation, which represents one out of multiple forms of post-translational modifications, FPP (C_{15}) and GGPP (C_{20}), respectively, represent the activated forms of the farnesyl and geranylgeranyl units that are covalently attached to the cysteine residue of a distinct tetrapeptide motif (CaaX) of many members of the Ras protein superfamily.[18] The prenyl side chain mediates membrane association, which is essential for Ras protein biologic activity. In addition, γ proteins of heterotrimeric G proteins ($G_{\alpha\beta\gamma}$), which are activated by G protein-coupled receptors, are also subject to farnesylation (γ1) or geranylgeranylation (γ2).[19] Ras activates not only the MAPK signaling cascade but also the phosphoinositide 3-kinase (PI3K)-AKT-mTOR pathway (Fig. 2).[6] Signaling through this pathway is essential not only for glycolytic metabolism[20] but also for the lipogenic program.[21] mTOR promotes glycolysis, which is prerequisite for the accumulation of cytosolic citrate and AKT stimulates the conversion of citrate into acetyl-CoA by phosphorylating ACLY.[9] Abundant cytosolic acetyl-CoA then fuels mTOR/SREBP-driven mevalonate metabolism and the resulting accumulation of FPP (or GGPP) facilitates prenylation of Ras, thus also generating a feed forward loop (Fig. 2).

In experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis, GGPP has been shown to be crucial for proliferation, whereas both GGPP and FPP regulated type 1 T helper (Th1) cell differentiation of myelin-reactive T cells.[22] Specifically, geranylgeranylated RhoA and farnesylated Ras have been implicated in proliferative and cytokine responses of these autoreactive T cells. Likewise, inhibition of farnesylation has been shown to impair cytokine production in murine Th1 and Th2 T cell clones.[23]

A distinct form of prenylation is also required to maintain ATP generation through oxidative phosphorylation (OXPHOS). Coenzyme Q (CoQ or ubiquinone) serves as a diffusible, lipid-soluble electron shuttle between large, relatively immobile macromolecular complexes in the electron transport chain at the inner mitochondrial membrane, which is the site of OXPHOS in eukaryotes. In human CoQ_{10} (Fig. 1), the lipid membrane anchor is a decaprenyl side chain (C_{50}) consisting of 10 C_5 isoprenyl units.[6] Human prenyl (decaprenyl) diphosphate synthase, subunit 1 (PDSS1) (Fig. 1) catalyzes the elongation of GPP or FPP with several IPP moieties to form the polyisoprenoid chain, which is then attached to a 4-hydroxybenzoate ring. Therefore, efficient CoQ_{10} biosynthesis depends on the functionality of PDSS1 on the one hand, and on the

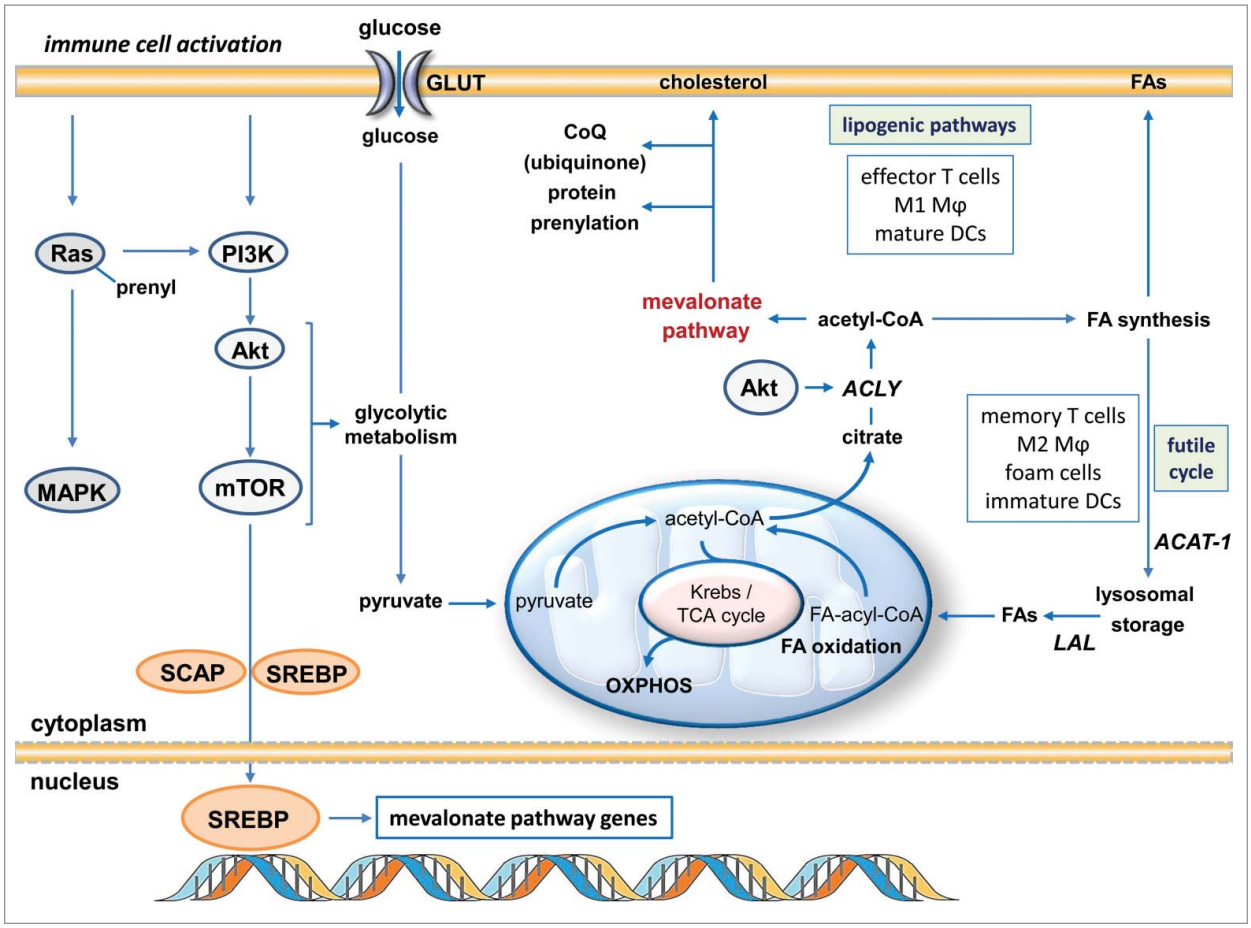

Figure 2. The metabolic reprogramming associated with immune cell activation engages the mevalonate pathway. Immune cell stimulation activates the PI3K-AKT-mTOR pathway and promotes uptake of glucose via glucose transporter (GLUT) proteins followed by glycolysis and oxidation of glucose-derived pyruvate in the mitochondrial tricarboxylic acid cycle (TCA) cycle, which drives oxidative phosphorylation (OXPHOS). Activated immune cells also export citrate to the cytosol, where it is converted back to acetyl-coenzyme A (acetyl-CoA) by ATP citrate lyase (ACLY). Cytosolic acetyl-CoA serves as a metabolic precursor not only for fatty acid (FA) synthesis and protein acetylation but also for mevalonate metabolism (the lipogenic pathways). mTOR signaling also leads to the activation of sterol regulatory element-binding proteins (SREBP), which are the main transcription factor for mevalonate pathway-associated genes in immune cells. In a feed-forward loop, enhanced mevalonate metabolism facilitates prenylation of Ras proteins and thus further enhances pathway activity. In an apparently futile cycle of concurrent synthesis and oxidation, *de novo* synthesized fatty acids (FA) are stored in the lysosome. Lysosomal acid lipase (LAL) catalyzes the release of these FA, which are shuttled into the mitochondria for ATP generation through ß-oxidation.

other, on the availability of its mevalonate-derived substrates IPP, GPP and FPP. Long-term therapy with statins, which inhibit the mevalonate pathway early on, may cause CoQ_{10} deficiency via PDSS1 substrate depletion with dramatic cardiac consequences, preferentially in elderly patients.[24] Conversely, PDSS1 inactivation due to mutations in the PDSS1 gene has been reported to cause clinically apparent OXPHOS disorders.[25] Collectively, these observations point toward the importance of mevalonate metabolism for OXPHOS.

Macrophages and dendritic cells

Macrophages can adopt distinct phenotypes, which either participate in immune responses against microbial pathogens and tumors (M1) or contribute to immunity against parasites as well as to tissue repair (M2).[26] Classical activation with interferon (IFN)-γ in combination with toll-like receptor (TLR) agonists such as lipopolysaccharide (LPS) generates M1 macrophages, whereas full M2 activation, also known as alternative activation, occurs in response to IL-4 and macrophage colony stimulating factor (M-CSF).[27] Classical activation of M1 macrophages involves the upregulation of GM-CSF production.[28] Importantly, GM-CSF but not M-CSF primes monocytes for enhanced responses to LPS.[29] Recent work has revealed the metabolic imprinting that underlies the GM-CSF priming effect. GM-CSF promotes glycolysis and concomitantly mevalonate metabolism by increasing 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in macrophages.[30] GM-CSF thus creates the metabolic requirements for the subsequent proinflammatory response induced by the TLR4 agonist LPS. Inhibition of both, glycolysis or HMG-CoA reductase, prevented the inflammatory effects of GM-CSF priming in macrophages. LPS has also been shown to boost glycolysis[31] and to stimulate the lipogenic pathway, which may in part be due to the induction of endogenous GM-CSF. In macrophages, lipogenesis is regulated by sterol regulatory element–binding protein 1a, which also directly activates inflammasome function resulting in caspase-1 mediated release of bioactive IL-1ß and IL-18.[32, 33] This finding established a strong link between the lipogenic program and inflammation. During macrophage activation, AKT can directly regulate mevalonate pathway activity by phosphorylating the isopentenyl diphosphate (IPP)-

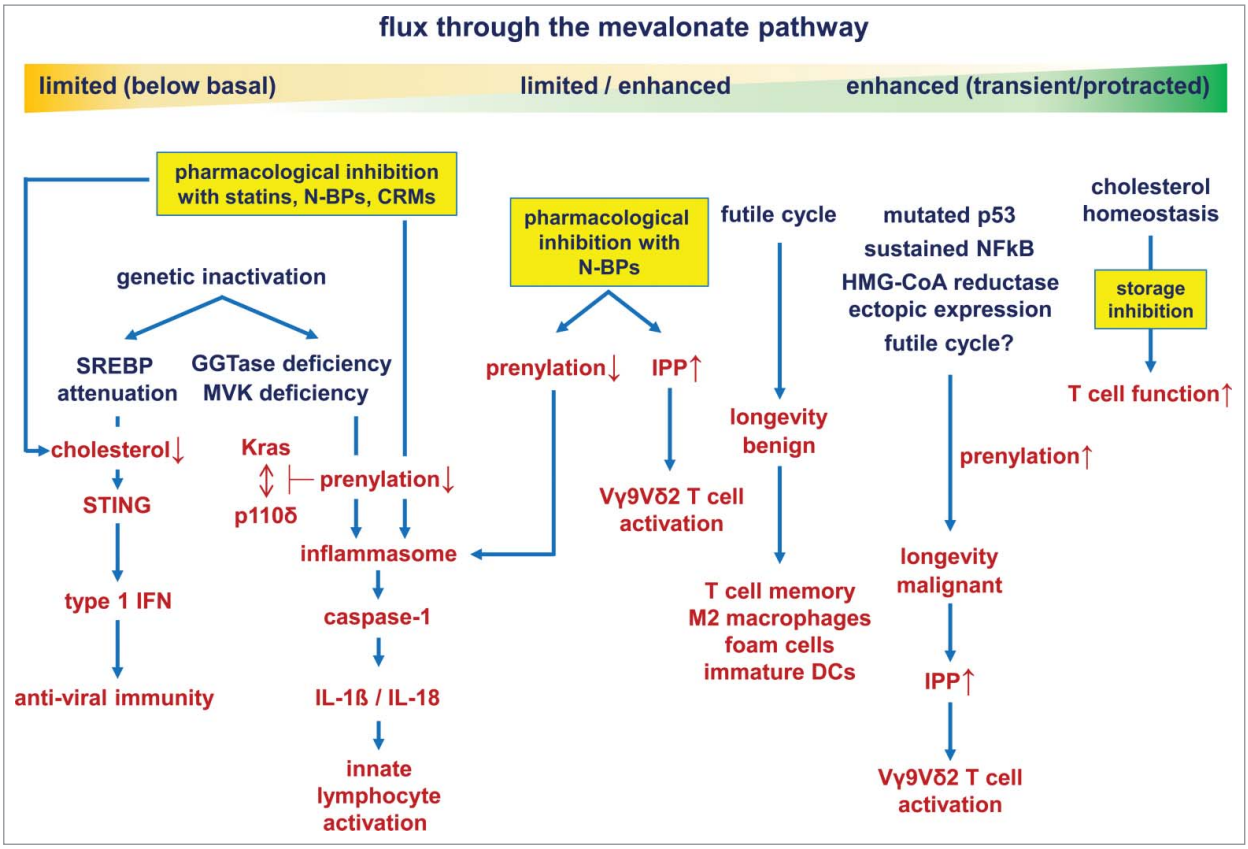

Figure 3. Inflammatory and immune responses to mevalonate pathway dysregulation. The responses induced by restricted flux have been studied using pharmacological inhibitors (statins and nitrogen-containing bisphosphonates, N-BPs), caloric restriction mimetics (CRM) such as hydroxycitrate (see also Fig. 1) or by genetic inactivation of geranylgeranyltransferase (GGTase), mevalonate kinase (MVK) or SREBP cleavage-activating protein (SCAP). Enhanced or uncontrolled flux can result from gain-of-function p53 mutation, sustained NFkB activation associated with chronic inflammation, ectopic expression of HMG-CoA reductase, or possibly also by futile metabolic constellations. Cell longevity resulting from sustained mevalonate metabolism and protein prenylation may physiologically be important for T cell memory establishment or pathologically manifest as malignant transformation. Among the various tools currently available for mevalonate pathway manipulation, N-BPs are unique, since they increase levels of IPP and simultaneously inhibit protein prenylation. Vγ9Vδ2 T cells, which are activated by increased levels of IPP and other mevalonate pathway intermediates, are intended to perform broad immune surveillance of enhanced mevalonate metabolism.

generating enzyme MVD (Fig. 1).[34] The resulting increase in mevalonate metabolism facilitated Rac1 activation, which requires prenylation, and thus promoted macrophage survival.[34] Specific evidence for the importance of mevalonate metabolism in inflammation was obtained in numerous studies showing that pharmacological mevalonate pathway inhibition can modulate a broad range of pro-inflammatory mechanisms by suppressing protein prenylation.[35] Collectively, these findings clearly indicate that classical activation of M1 macrophages is associated with enhanced mevalonate metabolism (Fig. 2).

Alternative (M2) activation is driven by IL-4,[26] an anti-inflammatory cytokine that suppresses macrophage development and M1 activation.[36, 37] M-CSF synergizes with IL-4 to support full M2 activation in an mTORC2-mediated pathway.[27] In contrast to GM-CSF, M-CSF is unable to prime macrophages for enhanced responses to LPS through metabolic reprogramming.[29, 30] Surprisingly, M2 activation has recently been found to also depend on glycolysis, however it appears to be a feature of M2 macrophages that glucose metabolism in M2 cells specifically increases FA oxidation to support OXPHOS, and these processes appear to be critical for full M2 activation.[27] An interesting and, at first glance, futile metabolic aspect of full M2 activation is the concurrent operation of FA synthesis and FA oxidation. However, increasing evidence supports the view that FA synthesis is required to effectively fuel FA oxidation.[38, 39] Together, these findings indicated that glycolysis primarily fuels the lipogenic program including mevalonate metabolism in M1 macrophages (Fig. 2). In contrast, glucose metabolism increasingly fuels FA synthesis for enhanced FA oxidation to drive OXPHOS in M2 macrophage activation. Such a metabolic constellation may limit mevalonate pathway activity, which likewise depends on acetyl-CoA, and thus prevent pro-inflammatory responses.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs).[40] In steady-state, DCs are immature with poor T cell stimulatory capacity and instead sample the microenvironment for the presence of pathogens. In response to pro-inflammatory signals, DCs undergo an activation program that has also been referred to as maturation, which is characterized by the downregulation of antigen uptake capacity and upregulation of the machinery related to T cell activation. DC development from monocytes under inflammatory situations depends on GM-CSF, and LPS can activate these DCs suggesting that the metabolic regulation of DC maturation might be similar to M1 macrophage activation and therefore also requires high flux through the mevalonate pathway (Fig. 2). DCs differentiated from bone marrow in the presence of GM-CSF have been

used as a model for inflammatory monocyte-derived DCs.[41] LPS has been shown to induce early glycolysis in such DCs within minutes, which feeds the TCA cycle, however, not for OXPHOS and ATP generation but for enhanced citrate production and export into the cytosol, where ACLY converts citrate back into acetyl-CoA for lipogenesis.[41] LPS-induced *de novo* FA synthesis in DCs has been shown to be important for the generation of additional membranes to expand the endoplasmic reticulum (ER) and the Golgi. Since cholesterol is a vital component of these membranes, LPS-stimulated membrane biogenesis is likely to depend on mevalonate metabolism. *De novo* lipogenesis may also support the generation of veil-like membrane protrusions, which constitute the typical dendritic morphology of mature DCs. Whereas numerous veils provide abundant contact surface for lymphocyte activation, expanded ER and Golgi ensures the secretory capacity of mature T cell stimulatory DCs. The membrane dynamics that participate in the formation and movement of veils depend on Rho GTPase and its effector Rho-associated protein kinase (ROCK), which are important regulators of the actin cytoskeleton.[42] Importantly, Rho must be geranylgeranylated to be able to interact with ROCK and to induce its activation. The vesicular transport that mediates secretion of inflammatory cytokines depends on Rho and Rab proteins,[43] which likewise require prenylation for their activation.[6] Taken together, DC maturation depends on a glycolysis-fueled lipogenic program engaging both, FA synthesis and mevalonate metabolism (Fig. 2).

Monocyte-derived DCs (MoDCs) that develop in the presence of GM-CSF and IL-4[44] have been shown to produce substantial amounts of M-CSF,[45] suggesting that these cells might display a mixed metabolic profile combining aspects of M1 and M2 macrophages (Fig. 2).[27] This particular metabolic constellation may be required to enable DC differentiation through IL-4 mediated inhibition of macrophage activation[37] on the one hand, and on the other, to concomitantly maintain the GM-CSF mediated responsiveness to pro-inflammatory stimuli such as LPS[30] that induce the further differentiation of these cells into fully mature immunogenic DCs.[46]

Collectively, these observations suggest the view that resting conditions (homeostasis) only support core mevalonate metabolism because acetyl-CoA is preferentially used for FA synthesis to fuel FA oxidation for OXPHOS and ATP generation. In contrast, inflammation boosts aerobic glycolysis that translates into enhanced mevalonate metabolism for cholesterol synthesis and protein prenylation in DCs and macrophages. From a metabolic point of view, classical (M1) macrophage activation and DC maturation may therefore be considered pro-inflammatory effector responses comparable to that of $CD8^+$ T cells, since they critically depend on mTOR-driven glycolytic capacity and high flux through the mevalonate pathway (Fig. 2).

The futile cycle and mevalonate metabolism

A distinct metabolic constellation revolving around acetyl-CoA has recently been described for various immune cells. Memory T cells appear to operate an apparently futile cycle of concurrent FA synthesis and FA oxidation (Fig. 2) to maintain long-term survival.[38] *De novo* synthesized FAs are stored in the lysosome. Lysosomal acid lipase catalyzes the release of these FAs, which are shuttled into the mitochondria for ATP generation through ß-oxidation. Recent findings by Guijas et al. suggest that foam cells,[39] which are considered a hallmark of the atherosclerotic lesion, engage a similar or possibly the same futile cycle to ensure their own survival, thus driving the atherogenic process. As outlined above, M2 macrophages were also found to combine FA synthesis and FA oxidation.[27] FA synthesis is obviously required to effectively fuel FA oxidation to drive OXPHOS. This observation made in T cells and macrophages raised the question of whether the longevity of other cells such as transformed cells including cancer-initiating cells is a consequence of this particular futile metabolism (Fig. 3).[47, 48] Importantly, the acetyl-CoA resulting from FA breakdown becomes available not only for FA biosynthesis - to drive the futile cycle - but also for the survival-promoting mevalonate pathway.[6] Sustained mevalonate metabolism, although at a relatively low level, may facilitate prolonged protein prenylation and may thus promote cell longevity, suggesting that FA metabolism might also be an attractive target to manipulate mevalonate metabolism in cancer therapies.

Immune responses to limited flux

Given the importance of mevalonate pathway products in multiple aspects of cell biology, it is reasonable to assume that a lack of mevalonate metabolism is hardly compatible with cell survival and tissue homeostasis.[49] Conditions of limited flux can be simulated by inhibiting critical enzymatic steps of the biosynthetic route using drugs or by inactivation or attenuation of the corresponding genes. In addition, an autosomal recessive metabolic disorder, referred to as hyper immunoglobulin D syndrome turned out to be useful for studying the consequences of insufficient mevalonate pathway activity. In these patients, mevalonate kinase (MVK) activity is reduced to 5 to 15 % of normal due to a loss-of-function mutation in the MVK gene (Fig. 1).[50] A recurrent observation in these studies was that limited flux through the mevalonate pathway did not simply result in cell death by apoptosis, which would be immunologically silent, but instead could also trigger inflammatory and immune responses.

Immunogenic cell death (ICD)-inducing cancer therapy based on anthracyclines or oxaliplatin has been demonstrated to elicit antitumor immune responses that substantially contribute to the long-term success of these chemotherapy regimens.[51] Autophagy is indispensable for ICD, because autophagy ensures release of ATP into the extracellular space and recruits APCs into the close vicinity of dying tumor cells. Hydroxycitrate (HC) is a drug candidate (a caloric restriction mimetic, CRM), which mimics the biochemical effects of starvation.[52] By competitively inhibiting ACLY (Figs. 1 and 2), HC reduces the availability of cytoplasmic acetyl-CoA and thus limits mevalonate metabolism as well as protein acetylation, both conditions that increase autophagic flux.[53, 54] In this manner, HC has recently been shown to improve ICD-inducing chemotherapy in a T cell-dependent fashion and this effect involved the depletion of tumor-infiltrating regulatory T cells. Importantly, the stimulatory effect of HC could only be observed in the treatment of autophagy-competent tumors.[52]

Statins, which are a widely prescribed class of drugs for the treatment of cardiovascular disease, inhibit the pathway early

on (Figs. 1 and 3) and thus cause general depletion of mevalonate and its downstream metabolites.[53] In contrast, nitrogen-containing bisphosphonates (N-BPs), which are used to prevent bone resorption in osteoporosis and malignant bone disease, inhibit mevalonate metabolism at the level of FPP synthase and generate a twofold effect: upstream accumulation of IPP as well as downstream depletion of FPP and GGPP (Figs. 1 and 3).[6] The inhibitory effect of N-BPs on protein prenylation in osteoclasts is the major mechanism of N-BP mediated prevention of bone degradation. However, the metabolic perturbation resulting from statin or N-BP treatment can also lead to inflammasome and caspase-1 activation in DCs.[55, 56] Caspase-1 proteolytically processes the inactive proforms of IL-1ß and IL-18, which can then be secreted as mature cytokines with strong costimulatory activity for innate lymphocytes (Fig. 3).[57] In the presence of IL-2, both statins and N-BPs could induce innate lymphocyte activation via caspase-1 mediated cytokine maturation (Fig. 3).[55, 56] Zoledronic acid, the most potent N-BP currently available for clinical use, has also been shown to restore doxorubicin chemosensitivity and immunogenic cell death in otherwise multidrug-resistant human cancer cells.[58]

Macrophages that fail to perform protein geranylgeranylation due to a deficiency of geranylgeranyl transferase-I (GGTase-I) have been shown to be hyper-activated by lipopolysaccharide and mice with conditional deficiency in GGTase-I in myeloid cells develop inflammatory arthritis spontaneously.[59] Mechanistic studies performed with GGTase-I deficient mice revealed that limited flux through the mevalonate pathway decreased the production of GGPP and attenuated protein geranylgeranylation. Since protein geranylgeranylation positively regulates the interaction of the Kras GTPase with the PI3K catalytic subunit p110δ (Figs. 2 and 3), impaired geranylgeranylation prevented PI3K activation. Surprisingly, disturbed interaction of Kras with p110δ resulted in hyper-inflammatory conditions, including constitutive IL-1ß release from macrophages, elevated expression of pyrin protein and spontaneous activation of the pyrin inflammasome (Fig. 3).[59]

In another recent study, the immunological consequences of limited mevalonate pathway activity have been investigated in mice with macrophage-specific deletion of SCAP. Upon genetic deletion of SCAP, SREBP2 transcriptional activities are significantly attenuated, resulting in markedly reduced expression of mevalonate pathway genes (Figs. 1-3).[60] The authors reported that a reduced cholesterol pool size resulting from limited flux through the mevalonate pathway could spontaneously induce a type I IFN response in a stimulator of interferon genes (STING, TMEM173)-dependent fashion.[60] Cholesterol supplementation was able to normalize type I IFN levels by preventing STING signaling in SREBP2-deficient macrophages. Intriguingly, this lipid metabolic-inflammatory circuit facilitated the development of anti-viral immunity (Fig. 3).

Similar to the observations made with statins, lack of MVK activity (Figs. 1 and 3) in hyper immunoglobulin D syndrome (MVK deficiency) also resulted in downstream depletion of GGPP and reduced geranylgeranylation of small GTPases (Rho, Rac, Rap) in patient peripheral blood mononuclear cells followed by inflammasome-dependent caspase-1 activation and release of mature IL-1ß,[61] which is known to be pivotal to the pathogenesis of most of the periodic fever syndromes.[50]

Immune responses to enhanced flux

Enhanced flux through the mevalonate pathway can lead to malignant transformation.[62] In breast cancer cells, protein geranylgeranylation has been shown to be required for the malignant, invasive phenotype caused by mutant p53, which enhances mevalonate metabolism instead of suppressing it (Fig. 3).[63] Moreover, ectopic expression of HMG-CoA reductase, which catalyzes the first committed step of mevalonate metabolism, enhances growth of transformed and nontransformed breast cells under anchorage-independent conditions or as xenografts in immunocompromised mice and, importantly, cooperates with prenylated RAS (Fig. 2) to drive the transformation of primary mouse embryonic fibroblasts cells.[64] The epidemiologic observation that statins can lower the risk of certain cancers[65] can be best explained by statin-mediated attenuation of protein prenylation.

In addition to a general increase in flux through the mevalonate pathway, individual branches of the pathway may be fueled preferentially, for instance as a consequence of redistribution of isoprenoid precursors. Chronic inflammation has been shown to inhibit hepatic cholesterol conversion into bile acids, causing a redistribution of metabolites to other branches of the mevalonate pathway (Fig. 1).[66] The resulting accumulation of FPP and GGPP facilitated protein prenylation, especially geranylgeranylation of a Rho GTPase.[66] This finding may also provide an explanation - at the metabolic level – for the well-established link between inflammation and cancer development (Fig. 3).[67]

Surveillance of permanently enhanced mevalonate metabolism by Vγ9Vδ2 T cells

A potentially oncogenic mevalonate pathway obviously requires surveillance. Ectopic expression of HMG-CoA reductase not only drives malignant transformation[64] but also endows transfected B lymphoblasts (Daudi) with the capacity to activate Vγ9Vδ2 T cells (Fig. 3),[68] which are the most abundant population of $\gamma\delta$ T cells in human blood.[69] Although Vγ9Vδ2 T cells display remarkable functional plasticity, a main function of this subset is obviously to perform strong cytotoxic responses against infected and transformed cells and to produce effector cytokines with innate-like kinetics.[69, 70] Vγ9Vδ2 T cells have recently also been identified as a major source of IL-9, a pleiotropic cytokine also contributing to antitumor immunity.[71] The TCR of these unconventional T cells acts as a pattern recognition receptor and responds to the mevalonate-derived diphosphate-containing C_5 to C_{20} isoprenoids (IPP, DMAPP, GPP, FPP, GGPP) (Fig. 1), which are also referred to as phosphoantigens (pAgs) in $\gamma\delta$ T cell biology.[72, 73] As compared with the frequencies of bacteria- or virus-specific $\alpha\beta$ T cells, which range from 5 to 170 per 10^6 naïve T cells,[74] Vγ9Vδ2 T cells with their semi-invariant, pAg-reactive TCRs represent 5.000 to 50.000 per 10^6 T cells in peripheral blood during homeostasis and up to 60% of all T cells during infection[69] indicating that pAgs constitute strong danger signals and emphasizing the importance of immune surveillance of mevalonate metabolism.

A pharmacological strategy to induce intracellular accumulation of mevalonate-derived pAgs is the use of N-BPs, which not only inhibit protein prenylation, but also induce upstream accumulation of mevalonate metabolites (Figs. 1 and 3). The N-BP induced increase of the FPP synthase substrate IPP in antigen-presenting cells or other cells induces activation of Vγ9Vδ2 T cells.[68] This side effect of N-BPs, which was detected more incidentally, when patients with bone disease had increased frequencies of Vγ9Vδ2 T cells after their first N-BP administration,[75] indicated for the first time that accumulation of mevalonate pathway intermediates alerts Vγ9Vδ2 T cells.

Extracellular mevalonate metabolism

Numerous studies have demonstrated that exogenous (i.e. synthetic) pAgs can activate Vγ9Vδ2 T cells,[71, 72] however, they concomitantly raised the question of whether and how pAgs may be released from cells to accumulate in the extracellular space under physiologic conditions. Castella and colleagues have recently addressed this important issue and provided evidence that the cholesterol efflux transporter ABCA1 (ATP-binding cassette transporter A1) is involved in the export of intracellular pAgs from DCs[76] (Fig. 4). ABCA1 is well known to mediate the efflux of cholesterol, which is actually made from IPP (Fig. 1).[6] Castella et al. further demonstrated that apoA-I is required for Vγ9Vδ2 T cell activation through extracellular pAgs in a butyrophilin 3A1 (BTN3A1)-dependent manner (Fig. 4). BTN3A1 has been reported to bind pAgs and mediate activation of Vγ9Vδ2 T cells.[77, 78] To facilitate this process, BTN3A1 appears to be physically associated with ABCA1.[76] The work by Castella et al. also represents a valuable contribution to an ongoing debate in the field regarding 2 different mechanistic models of Vγ9Vδ2 T cell activation by pAgs (Fig. 4). In the allosteric model proposed by Harly et al., the interaction between intracellular pAgs and the intracellular B30.2 domain of BTN3A1 induces conformational changes of the extracellular domain, which are sensed by Vγ9Vδ2 T cells leading to their activation.[78] In the antigen presenting model put forward by Vavassori et al., intracellular pAgs are exported to the extracellular microenvironment by an "unidentified plasma membrane-associated transporter" and presented to Vγ9Vδ2 T cells by the BTN3A1 extracellular domain.[77] According to the findings of Castella et al., ABCA1 has now been identified as a crucial pAg exporter.[76] The 2 mechanisms may indeed cooperate to increase the degree of flexibility. Thus, both intracellular and extracellular pAg accumulation can be sensed and import-export mechanisms may ensure Vγ9Vδ2 T cell activation by either the allosteric or the presentation mechanism (Fig. 4).

The finding that pAgs are exported into the extracellular space for BTN3A1-mediated presentation to Vγ9Vδ2 T cells[76] calls for controlling mechanisms, since active export or lytic release of isoprenoid-derived pAgs from dysregulated or lysed cells into the extracellular space may lead to apoA-I mediated binding of pAgs to BTN3A1 on healthy adjacent tissue, thus causing collateral damage. We have recently shown that the ecto-ATPase CD39 also exhibits isoprenoid diphosphate phosphohydrolase activity[79] and may hence be involved in surveillance of this process. CD39 expressed by Vγ9Vδ2 T cells, T_{reg} cells, or other cells may inactivate secreted pAgs by dephosphorylation (Fig. 4) to avoid such side effects and ensure the specific elimination of the dysregulated cells.[79]

Transcellular lipid metabolism refers to a particular form of short distance intercellular communication, in which a lipid intermediate synthesized and released by one cell type, can be incorporated and further metabolized by another cell type. Such interaction between different cell types by shared metabolism is a well-described phenomenon during eicosanoid biosynthesis.[80] In accordance with such a concept of transcellular lipid metabolism, extracellular pAgs were previously shown to have the ability to enter cells and feed into mevalonate metabolism. In add-back experiments, drug-induced mevalonate pathway inhibition was restored by exogenous pAgs. Thus, addition of FPP, or more often of GGPP, was able to reinstate protein prenylation during statin-mediated inhibition of mevalonate metabolism (Fig. 1).[6, 22, 63] While pAg uptake could undoubtedly be documented, the mechanisms of internalization remained less clear. CD36, originally referred to as fatty acid

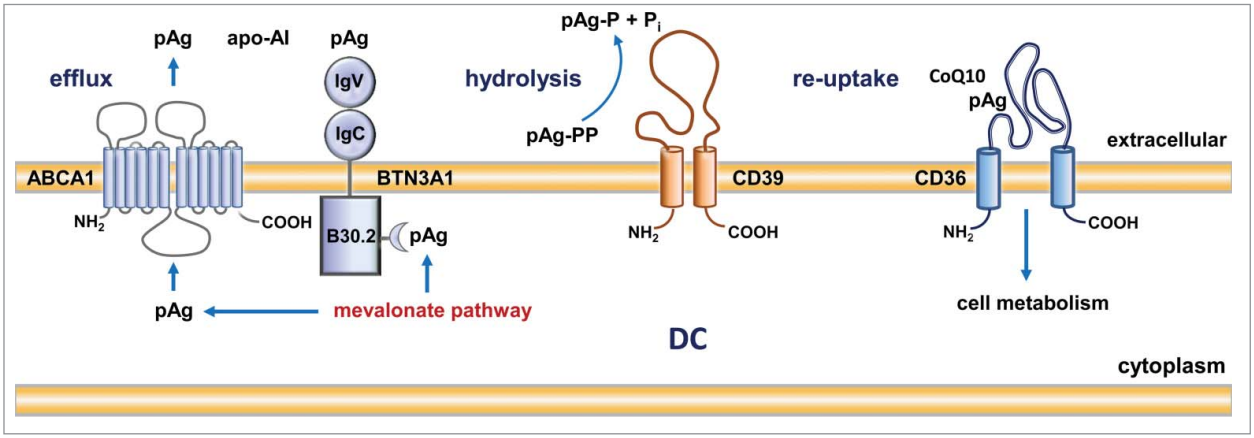

Figure 4. Extracellular mevalonate metabolism. Intracellular accumulation of mevalonate derivatives triggers homeostatic mechanisms leading to their export into the extracellular space. In dendritic cells (DC), the ATP-binding cassette transporter A1 (ABCA1) not only mediates efflux of cholesterol, which arises from IPP, but also exports IPP itself, which acts as an agonist of Vγ9Vδ2 T cells ("phosphoantigen," pAg). In the extracellular environment, pAgs can bind to apoA-I, which facilitates pAg presention by butyrophilin 3A1 (BTN3A1) on the DC surface to Vγ9Vδ2 T cells. The ecto-ATPase CD39 also exhibits intrinsic isoprenoid diphosphate phosphohydrolase activity and may control strength and duration of antigen presentation through the hydrolytic inactivation of pAgs. Finally, scavenger receptors such as CD36 may internalize not only IPP-derived CoQ_{10} but also IPP itself.

translocase and a defining member of the class B scavenger receptor family, has now been identified as a likely candidate. CD36 is a multifunctional receptor that also participates in a range of processes unrelated to fatty acid uptake. Recently, CD36 has been shown to also mediate cellular uptake of coenzyme Q_{10} (CoQ_{10}),[81] thus implicating CD36 in the internalization of extracellular isoprenoids (Fig. 4). In human CoQ_{10} (Fig. 1), the lipid membrane anchor is a decaprenyl side chain (C_{50}) consisting of 10 C_5 isoprenyl units.[6] By analogy with IPP efflux,[76] which can be mediated by a transporter of IPP-derived cholesterol, IPP uptake may be performed by a scavenger receptor known to incorporate IPP-derived CoQ_{10}.[81] Given that CD36 may mediate the internalization of isoprenoids,[81] dephosphorylation of pAgs by CD39,[79] which also increases pAg lipophilicity, may facilitate uptake and recycling of pAgs.[82]

Concluding remarks and perspectives

To survive and maintain core functions during homeostasis, immune cells have to ensure basal flux through the mevalonate pathway. In contrast, high flux is transiently required to provide important products such as cholesterol or to enable protein prenylation for the realization of diverse cellular functions ranging from proliferation and migration to cytokine production and cytotoxic degranulation during an immune response. Recent work has convincingly demonstrated that both, restricted flux (below basal flux) as well as permanently increased flux through the mevalonate pathway triggers alarms and leads to distinct inflammatory and immune responses. Intriguingly, evidence has also been obtained that such immune responses resulting from metabolic dysregulation may be harnessed to improve cancer immunotherapy. Manipulation of mevalonate pathway activity may be very effective in attempts to awake latent immunosurveillance for cancer therapy. Checkpoint inhibition blockade,[83] ICD-inducing chemotherapy,[51] or any type of tumor vaccine, for instance based on DCs,[84] could be combined with mevalonate pathway manipulation to generate synergistic effects. Promising data have already been obtained in murine cancer models showing that ACLY inhibition can enhance immunogenic chemotherapy[52] and ACAT-1 inhibition was effective in generating better T cells in the context of checkpoint inhibitor-based immunotherapy.[16, 17] Finally, N-BPs may play a special role, since they increase tumor cell immunogenicity and concomitantly recruit Vγ9Vδ2 T cells, which are poised to perform strong cytotoxic antitumor responses.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the Austrian Science Fund (FWF; P 28923-B28).

References

1. Motulsky AG. The 1985 Nobel Prize in physiology or medicine. Science 1986; 231:126-9; PMID:3510453; https://doi.org/10.1126/science.3510453
2. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. Nat Rev Immunol 2016; 16:553-65; PMID:27396447; https://doi.org/10.1038/nri.2016.70
3. Murray PJ, Rathmell J, Pearce E. SnapShot: Immunometabolism. Cell Metab 2015; 22:190-e1; PMID:26154058; https://doi.org/10.1016/j.cmet.2015.06.014
4. Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. J Exp Med 2015; 212:1345-60; PMID:26261266; https://doi.org/10.1084/jem.20151159
5. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. Annu Rev Immunol 2013; 31:259-83; PMID:23298210; https://doi.org/10.1146/annurev-immunol-032712-095956
6. Thurnher M, Gruenbacher G. T lymphocyte regulation by mevalonate metabolism. Sci Signal 2015; 8:re4; PMID:25829448; https://doi.org/10.1126/scisignal.2005970
7. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature 1990; 343:425-30; PMID:1967820; https://doi.org/10.1038/343425a0
8. Osinalde N, Mitxelena J, Sanchez-Quiles V, Akimov V, Aloria K, Arizmendi JM, Zubiaga AM, Blagoev B, Kratchmarova I. Nuclear phosphoproteomic screen uncovers ACLY as mediator of IL-2-induced proliferation of CD4+ T lymphocytes. Mol Cell Proteomics 2016; 15:2076-92; PMID:27067055; https://doi.org/10.1074/mcp.M115.057158
9. Berwick DC, Hers I, Heesom KJ, Moule SK, Tavare JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. J Biol Chem 2002; 277:33895-900; PMID:12107176; https://doi.org/10.1074/jbc.M204681200
10. Zeng H, Chi H. mTOR and lymphocyte metabolism. Curr Opin Immunol 2013; 25:347-55; PMID:23722114; https://doi.org/10.1016/j.coi.2013.05.002
11. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012; 149:274-93; PMID:22500797; https://doi.org/10.1016/j.cell.2012.03.017
12. Brown MS, Goldstein JL. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997; 89:331-40; PMID:9150132; https://doi.org/10.1016/S0092-8674(00)80213-5
13. Ricoult SJ, Yecies JL, Ben-Sahra I, Manning BD. Oncogenic PI3K and K-Ras stimulate de novo lipid synthesis through mTORC1 and SREBP. Oncogene 2016; 35:1250-60; PMID:26028026; https://doi.org/10.1038/onc.2015.179
14. Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, Shih R, Parks JS, Edwards PA, Jamieson BD et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. Cell 2008; 134:97-111; PMID:18614014; https://doi.org/10.1016/j.cell.2008.04.052
15. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, Marbois BN, Komisopoulou E, Wilson EB, Osborne TF et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. Nat Immunol 2013; 14:489-99; PMID:23563690; https://doi.org/10.1038/ni.2570
16. Yang W, Bai Y, Xiong Y, Zhang J, Chen S, Zheng X, Meng X, Li L, Wang J, Xu C et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature 2016; 531:651-5; PMID:26982734; https://doi.org/10.1038/nature17412
17. Kidani Y, Bensinger SJ. Modulating cholesterol homeostasis to build a better T cell. Cell Metab 2016; 23:963-4; PMID:27304495; https://doi.org/10.1016/j.cmet.2016.05.015
18. Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. J Cell Sci 2005; 118:843-6; PMID:15731001; https://doi.org/10.1242/jcs.01660
19. Marrari Y, Crouthamel M, Irannejad R, Wedegaertner PB. Assembly and trafficking of heterotrimeric G proteins. Biochemistry 2007; 46:7665-77; PMID:17559193; https://doi.org/10.1021/bi700338m
20. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF, Powell JD The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol 2011; 12:295-303; PMID:21358638; https://doi.org/10.1038/ni.2005

21. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab 2008; 8:224-36; PMID:18762023; https://doi.org/10.1016/j.cmet.2008.07.007
22. Dunn SE, Youssef S, Goldstein MJ, Prod'homme T, Weber MS, Zamvil SS, Steinman L. Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. J Exp Med 2006; 203:401-12; PMID:16476765; https://doi.org/10.1084/jem.20051129
23. Marks RE, Ho AW, Robbel C, Kuna T, Berk S, Gajewski TF. Farnesyltransferase inhibitors inhibit T-cell cytokine production at the post-transcriptional level. Blood 2007; 110:1982-8; PMID:17545504; https://doi.org/10.1182/blood-2006-06-031088
24. Harper CR, Jacobson TA. The broad spectrum of statin myopathy: From myalgia to rhabdomyolysis. Curr Opin Lipidol 2007; 18:401-8; PMID:17620856; https://doi.org/10.1097/MOL.0b013e32825a6773
25. Mollet J, Giurgea I, Schlemmer D, Dallner G, Chretien D, Delahodde A, Bacq D, de Lonlay P, Munnich A, Rötig A. Prenyldiphosphate synthase, subunit 1 (PDSS1) and OH-benzoate polyprenyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders. J Clin Invest 2007; 117:765-72; PMID:17332895; https://doi.org/10.1172/JCI29089
26. Biswas SK, Mantovani A. Orchestration of metabolism by macrophages. Cell Metab 2012; 15:432-7; PMID:22482726; https://doi.org/10.1016/j.cmet.2011.11.013
27. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, Pearce EJ. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. Immunity 2016; 45:817-30; PMID:27760338; https://doi.org/10.1016/j.immuni.2016.09.016
28. Lee MT, Kaushansky K, Ralph P, Ladner MB. Differential expression of M-CSF, G-CSF, and GM-CSF by human monocytes. J Leukoc Biol 1990; 47:275-82; PMID:1689760.
29. Hayes MP, Enterline JC, Gerrard TL, Zoon KC. Regulation of interferon production by human monocytes: Requirements for priming for lipopolysaccharide-induced production. J Leukoc Biol 1991; 50:176-81; PMID:1649241
30. Na YR, Gu GJ, Jung D, Kim YW, Na J, Woo JS, Cho JY, Youn H, Seok SH. GM-CSF induces inflammatory macrophages by regulating glycolysis and lipid metabolism. J Immunol 2016; 197:4101-9; PMID:27742831; https://doi.org/10.4049/jimmunol.1600745
31. Feingold KR, Shigenaga JK, Kazemi MR, McDonald CM, Patzek SM, Cross AS, Moser A, Grunfeld C. Mechanisms of triglyceride accumulation in activated macrophages. J Leukoc Biol 2012; 92:829-39; PMID:22753953; https://doi.org/10.1189/jlb.1111537
32. Im SS, Yousef L, Blaschitz C, Liu JZ, Edwards RA, Young SG, Raffatellu M, Osborne TF. Linking lipid metabolism to the innate immune response in macrophages through sterol regulatory element binding protein-1a. Cell Metab 2011; 13:540-9; PMID:21531336; https://doi.org/10.1016/j.cmet.2011.04.001
33. Feingold KR, Hardardottir I, Memon R, Krul EJ, Moser AH, Taylor JM, Grunfeld C. Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. J Lipid Res 1993; 34:2147-58; PMID:8301233
34. Larson-Casey JL, Murthy S, Ryan AJ, Carter AB. Modulation of the mevalonate pathway by akt regulates macrophage survival and development of pulmonary fibrosis. J Biol Chem 2014; 289:36204-19; PMID:25378391; https://doi.org/10.1074/jbc.M114.593285
35. Greenwood J, Steinman L, Zamvil SS. Statin therapy and autoimmune disease: From protein prenylation to immunomodulation. Nat Rev Immunol 2006; 6:358-70; PMID:16639429; https://doi.org/10.1038/nri1839
36. Jansen JH, Wientjens GJ, Fibbe WE, Willemze R, Kluin-Nelemans HC. Inhibition of human macrophage colony formation by interleukin 4. J Exp Med 1989; 170:577-82; PMID:2666563; https://doi.org/10.1084/jem.170.2.577
37. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. Potential antiinflammatory effects of interleukin 4: Suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. Proc Natl Acad Sci U S A 1989; 86:3803-7; PMID:2786204; https://doi.org/10.1073/pnas.86.10.3803
38. O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, Qiu J, Smith AM, Lam WY, DiPlato LM et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity 2014; 41:75-88; PMID:25001241; https://doi.org/10.1016/j.immuni.2014.06.005
39. Guijas C, Meana C, Astudillo AM, Balboa MA, Balsinde J. Foamy monocytes are enriched in cis-7-hexadecenoic fatty acid (16:1n-9), a possible biomarker for early detection of cardiovascular disease. Cell Chem Biol 2016; 23:689-99; PMID:27265749; https://doi.org/10.1016/j.chembiol.2016.04.012
40. Steinman RM. Decisions about dendritic cells: Past, present, and future. Annu Rev Immunol 2012; 30:1-22; PMID:22136168; https://doi.org/10.1146/annurev-immunol-100311-102839
41. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJ et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. Nat Immunol 2014; 15:323-32; PMID:24562310; https://doi.org/10.1038/ni.2833
42. Kobayashi M, Azuma E, Ido M, Hirayama M, Jiang Q, Iwamoto S, Kumamoto T, Yamamoto H, Sakurai M, Komada Y. A pivotal role of Rho GTPase in the regulation of morphology and function of dendritic cells. J Immunol 2001; 167:3585-91; PMID:11564770; https://doi.org/10.4049/jimmunol.167.7.3585
43. Stow JL. Nobel Prize discovery paves the way for immunological traffic. Nat Rev Immunol 2013; 13:839-41; PMID:24270771; https://doi.org/10.1038/nri3564
44. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM, Schuler G. Proliferating dendritic cell progenitors in human blood. J Exp Med 1994; 180:83-93; PMID:8006603; https://doi.org/10.1084/jem.180.1.83
45. Rieser C, Ramoner R, Bock G, Deo YM, Holtl L, Bartsch G, Thurnher M. Human monocyte-derived dendritic cells produce macrophage colony-stimulating factor: Enhancement of c-fms expression by interleukin-10. Eur J Immunol 1998; 28:2283-8; PMID:9710206; https://doi.org/10.1002/(SICI)1521-4141(199808)28:08%3c2283::AID-IMMU2283%3e3.0.CO;2-X
46. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: Downregulation by cytokines and bacterial products. J Exp Med 1995; 182:389-400; PMID:7629501; https://doi.org/10.1084/jem.182.2.389
47. Weinberg SE, Chandel NS. Futility sustains memory T cells. Immunity 2014; 41:1-3; PMID:25035944; https://doi.org/10.1016/j.immuni.2014.06.009
48. Thurnher M. Small drops get fat: Unexpected fatty acid in cytoplasmic lipid droplets. Cell Chem Biol 2016; 23:637-8; PMID:27341429; https://doi.org/10.1016/j.chembiol.2016.06.001
49. Berndt N, Hamilton AD, Sebti SM. Targeting protein prenylation for cancer therapy. Nat Rev Cancer 2011; 11:775-91; PMID:22020205; https://doi.org/10.1038/nrc3151
50. Drenth JP, van der Meer JW. Hereditary periodic fever. N Engl J Med 2001; 345:1748-57; PMID:11742050; https://doi.org/10.1056/NEJMra010200
51. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015; 28:690-714; PMID:26678337; https://doi.org/10.1016/j.ccell.2015.10.012
52. Pietrocola F, Pol J, Vacchelli E, Rao S, Enot DP, Baracco EE, Levesque S, Castoldi F, Jacquelot N, Yamazaki T et al. Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer Cell 2016; 30:147-60; PMID:27411589; https://doi.org/10.1016/j.ccell.2016.05.016
53. Thurnher M, Nussbaumer O, Gruenbacher G. Novel aspects of mevalonate pathway inhibitors as antitumor agents. Clin Cancer Res 2012; 18:3524-31; PMID:22529099; https://doi.org/10.1158/1078-0432.CCR-12-0489
54. Madeo F, Pietrocola F, Eisenberg T, Kroemer G. Caloric restriction mimetics: Towards a molecular definition. Nat Rev Drug Discov 2014; 13:727-40; PMID:25212602; https://doi.org/10.1038/nrd4391
55. Gruenbacher G, Gander H, Nussbaumer O, Nussbaumer W, Rahm A, Thurnher M. IL-2 costimulation enables statin-mediated activation of

human NK cells, preferentially through a mechanism involving CD56+ dendritic cells. Cancer Res 2010; 70:9611-20; PMID:20947520; https://doi.org/10.1158/0008-5472.CAN-10-1968
56. Nussbaumer O, Gruenbacher G, Gander H, Thurnher M. DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by gammadelta T lymphocytes. Blood 2011; 118:2743-51; PMID:21673342; https://doi.org/10.1182/blood-2011-01-328526
57. Martinon F, Tschopp J. Inflammatory caspases: Linking an intracellular innate immune system to autoinflammatory diseases. Cell 2004; 117:561-74; PMID:15163405; https://doi.org/10.1016/j.cell.2004.05.004
58. Riganti C, Massaia M. Inhibition of the mevalonate pathway to override chemoresistance and promote the immunogenic demise of cancer cells: Killing two birds with one stone. Oncoimmunology 2013; 2:e25770; PMID:24327936; https://doi.org/10.4161/onci.25770
59. Akula MK, Shi M, Jiang Z, Foster CE, Miao D, Li AS, Zhang X, Gavin RM, Forde SD, Germain G et al. Control of the innate immune response by the mevalonate pathway. Nat Immunol 2016; 17:922-9; PMID:27270400; https://doi.org/10.1038/ni.3487
60. York AG, Williams KJ, Argus JP, Zhou QD, Brar G, Vergnes L, Gray EE, Zhen A, Wu NC, Yamada DH et al. Limiting cholesterol biosynthetic flux spontaneously engages Type I IFN signaling. Cell 2015; 163:1716-29; PMID:26686653; https://doi.org/10.1016/j.cell.2015.11.045
61. Muller AL, Freed DH. Basic and clinical observations of mevalonate depletion on the mevalonate signalling pathway. Curr Mol Pharmacol 2017; 10(1):6-12; PMID:26758946; https://doi.org/10.2174/1874467209666160112125805
62. Mullen PJ, Yu R, Longo J, Archer MC, Penn LZ. The interplay between cell signalling and the mevalonate pathway in cancer. Nat Rev Cancer 2016; 16:718-31; PMID:27562463; https://doi.org/10.1038/nrc.2016.76
63. Freed-Pastor WA, Mizuno H, Zhao X, Langerod A, Moon SH, Rodriguez-Barrueco R, Barsotti A, Chicas A, Li W, Polotskaia A et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. Cell 2012; 148:244-58; PMID:22265415; https://doi.org/10.1016/j.cell.2011.12.017
64. Clendening JW, Pandyra A, Boutros PC, El Ghamrasni S, Khosravi F, Trentin GA, Martirosyan A, Hakem A, Hakem R, Jurisica I et al. Dysregulation of the mevalonate pathway promotes transformation. Proc Natl Acad Sci U S A 2010; 107:15051-6; PMID:20696928; https://doi.org/10.1073/pnas.0910258107
65. Graaf MR, Beiderbeck AB, Egberts AC, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. J Clin Oncol 2004; 22:2388-94; PMID:15197200; https://doi.org/10.1200/JCO.2004.02.027
66. Okin D, Medzhitov R. The effect of sustained inflammation on hepatic mevalonate pathway results in hyperglycemia. Cell 2016; 165:343-56; PMID:26997483; https://doi.org/10.1016/j.cell.2016.02.023
67. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010; 140:883-99; PMID:20303878; https://doi.org/10.1016/j.cell.2010.01.025
68. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. J Exp Med 2003; 197:163-8; PMID:12538656; https://doi.org/10.1084/jem.20021500
69. Chien YH, Meyer C, Bonneville M. gammadelta T Cells: First line of defense and beyond. Annu Rev Immunol 2014; 32:121-55; PMID:24387714; https://doi.org/10.1146/annurev-immunol-032713-120216
70. Ryan PL, Sumaria N, Holland CJ, Bradford CM, Izotova N, Grandjean CL, Jawad AS, Bergmeier LA. Pennington DJ Heterogeneous yet stable Vdelta2(+) T-cell profiles define distinct cytotoxic effector potentials in healthy human individuals. Proc Natl Acad Sci U S A 2016; 113:14378-83; PMID:27911793; https://doi.org/10.1073/pnas.1611098113
71. Peters C, Hasler R, Wesch D, Kabelitz D. Human Vdelta2 T cells are a major source of interleukin-9. Proc Natl Acad Sci U S A 2016; 113:12520-5; PMID:27791087; https://doi.org/10.1073/pnas.1607136113
72. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. Nature 1995; 375:155-8; PMID:7753173; https://doi.org/10.1038/375155a0
73. Gruenbacher G, Nussbaumer O, Gander H, Steiner B, Leonhartsberger N, Thurnher M. Stress-related and homeostatic cytokines regulate Vgamma9Vdelta2 T-cell surveillance of mevalonate metabolism. Oncoimmunology 2014; 3:e953410; PMID:25960933; https://doi.org/10.4161/21624011.2014.953410
74. Geiger R, Duhen T, Lanzavecchia A, Sallusto F. Human naive and memory CD4+ T cell repertoires specific for naturally processed antigens analyzed using libraries of amplified T cells. J Exp Med 2009; 206:1525-34; PMID:19564353; https://doi.org/10.1084/jem.20090504
75. Kunzmann V, Bauer E, Wilhelm M. Gamma/delta T-cell stimulation by pamidronate. N Engl J Med 1999; 340:737-8; PMID:10068336; https://doi.org/10.1056/NEJM199903043400914
76. Castella B, Kopecka J, Sciancalepore P, Mandili G, Foglietta M, Mitro N, Caruso D, Novelli F, Riganti C. Massaia M The ATP-binding cassette transporter A1 regulates phosphoantigen release and Vgamma9Vdelta2 T cell activation by dendritic cells. Nat Commun 2017; 8:15663; PMID:28580927; https://doi.org/10.1038/ncomms15663
77. Vavassori S, Kumar A, Wan GS, Ramanjaneyulu GS, Cavallari M, El Daker S, Beddoe T, Theodossis A, Williams NK, Gostick E et al. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human gammadelta T cells. Nat Immunol 2013; 14:908-16; PMID:23872678; https://doi.org/10.1038/ni.2665
78. Harly C, Guillaume Y, Nedellec S, Peigne CM, Monkkonen H, Monkkonen J, Li J, Kuball J, Adams EJ, Netzer S et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset. Blood 2012; 120:2269-79; PMID:22767497; https://doi.org/10.1182/blood-2012-05-430470
79. Gruenbacher G, Gander H, Rahm A, Idzko M, Nussbaumer O, Thurnher M. Ecto-ATPase CD39 Inactivates Isoprenoid-Derived Vgamma9Vdelta2 T Cell Phosphoantigens. Cell Rep 2016; 16:444-56; PMID:27346340; https://doi.org/10.1016/j.celrep.2016.06.009
80. Capra V, Rovati GE, Mangano P, Buccellati C, Murphy RC, Sala A. Transcellular biosynthesis of eicosanoid lipid mediators. Biochim Biophys Acta 2015; 1851:377-82; PMID:25218301; https://doi.org/10.1016/j.bbalip.2014.09.002
81. Anderson CM, Kazantzis M, Wang J, Venkatraman S, Goncalves RL, Quinlan CL, Ng R, Jastroch M, Benjamin DI, Nie B et al. Dependence of brown adipose tissue function on CD36-mediated coenzyme Q uptake. Cell Rep 2015; 10:505-15; PMID:25620701; https://doi.org/10.1016/j.celrep.2014.12.048
82. Crick DC, Andres DA, Waechter CJ. Novel salvage pathway utilizing farnesol and geranylgeraniol for protein isoprenylation. Biochem Biophys Res Commun 1997; 237:483-7; PMID:9299388; https://doi.org/10.1006/bbrc.1997.7145
83. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol 2015; 33:1974-82; PMID:25605845; https://doi.org/10.1200/JCO.2014.59.4358
84. Romero P, Banchereau J, Bhardwaj N, Cockett M, Disis ML, Dranoff G, Gilboa E, Hammond SA, Hershberg R, Korman AJ et al. The human vaccines project: A roadmap for cancer vaccine development. Sci Transl Med 2016; 8:334ps9; PMID:27075624; https://doi.org/10.1126/scitranslmed.aaf0685