

T lymphocyte regulation by mevalonate metabolism

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Whereas resting T cells, which have low metabolic requirements, use oxidative phosphorylation (OXPHOS) to maximize their generation of ATP, activated T cells, similar to tumor cells, shift metabolic activity to aerobic glycolysis, which also fuels mevalonate metabolism. Both sterol and nonsterol derivatives of mevalonate affect T cell function. The intracellular availability of sterols, which is dynamically regulated by different classes of transcription factors, represents a metabolic checkpoint that modulates T cell responses. The electron carrier ubiquinone, which is modified with an isoprenoid membrane anchor, plays a pivotal role in OXPHOS, which supports the proliferation of T cells. Isoprenylation also mediates the plasma membrane attachment of the Ras, Rho, and Rab guanosine triphosphatases, which are involved in T cell immunological synapse formation, migration, proliferation, and cytotoxic effector responses. Finally, multiple phosphorylated mevalonate derivatives can act as danger signals for innate-like $\gamma\delta$ T cells, thus contributing to the immune surveillance of stress, pathogens, and tumors. We highlight the importance of the mevalonate pathway in the metabolic reprogramming of effector and regulatory T cells.

Introduction

Signaling through the T cell receptor (TCR) serves as a paradigm for how cell surface receptors translate extrinsic cues into cellular responses (1). The metabolic regulation of T lymphocytes as a consequence of TCR stimulation has attracted much interest (2–5). It is increasingly appreciated that T cell activation results in metabolic reprogramming and that distinct T cell functions depend on the activation of appropriate metabolic pathways (2). In contrast to resting cells, which pursue efficient accumulation of adenosine triphosphate (ATP), activated T cells must ensure high metabolic flux through anabolic pathways. Stimulated T cells shift from using oxidative metabolism [oxidative phosphorylation (OXPHOS)] to aerobic glycolysis to meet these requirements because glycolysis produces many metabolites that can be used for biosynthetic purposes. Although reviews have discussed how a truncated tricarboxylic acid (TCA) cycle leads to increased amounts of cytosolic acetyl-coenzyme A (acetyl-CoA), a key metabolite for lipid biosynthesis, they have not fully addressed the growth-promoting mevalonate pathway, which is directly fed by accumulating acetyl-CoA, with its diverse classes of sterol and nonsterol isoprenoid products. Here, we focus on how mevalonate metabolites regulate T cells at many different levels, both as cell-intrinsic and cell-extrinsic metabolic cues.

Metabolic Regulation of T Cells: The Shift from OXPHOS to Aerobic Glycolysis

Quiescent T cells, like other nonproliferating cells, display relatively low levels of glycolytic activity, and they fully oxidize glucose-derived pyruvate or fatty acid-derived acetyl-CoA in the mitochondrial TCA cycle (Fig. 1) (2, 3). This process drives OXPHOS and maximizes the amount of ATP that can be generated from these substrates. The electron transport chain in the mitochondrion is the site of OXPHOS in eukaryotes. With energy derived from the TCA cycle, electron transfer from donors to acceptors through redox reactions is coupled with the transfer of protons across the inner mitochondrial membrane. The resulting electrochemical

proton gradient stimulates the generation of ATP from adenosine diphosphate by the enzyme ATP synthase.

In contrast, activated T cells have a substantially increased demand for metabolic resources because they must perform effector functions or accumulate biomass for cell growth and daughter cell generation (2, 3). Upon TCR stimulation, fatty acid β -oxidation is markedly decreased, and the amounts of glucose and amino acid transporters are increased on the T cell surface to facilitate nutrient uptake. The transcription factors c-Myc (6) and estrogen-related receptor α (7), which are increased in abundance during TCR-mediated activation, control metabolic reprogramming in T cells. Although activated T cells still engage in OXPHOS, glycolysis becomes the prevailing pathway even in the presence of oxygen, and it is therefore referred to as aerobic glycolysis, which is also known from early work in cancer biology as the Warburg effect. It has long been thought that the metabolic demands of T cell proliferation enforce the switch from OXPHOS to aerobic glycolysis. However, a study demonstrated that both OXPHOS and aerobic glycolysis can fuel $\alpha\beta$ T cell proliferation and survival, but only aerobic glycolysis supports full T cell effector function (8). Consistent with their rapid, innate-like responsiveness, $\gamma\delta$ T cells have greater amounts of glucose transporters on the cell surface than do $\alpha\beta$ T cells, they display enhanced glucose metabolism, and they exhibit effector function over a broader range of glucose concentrations than do $\alpha\beta$ T cells (9). It may be for this reason that $\gamma\delta$ T cells are less dependent on costimulation through the co-receptor CD28, which serves to enhance glycolytic activity in $\alpha\beta$ T cells (9).

In proliferating cells, glycolysis-derived pyruvate can enter a truncated TCA cycle. In this case, citrate is exported from the mitochondrion to the cytosol, where it is converted back to acetyl-CoA by ATP citrate lyase (10). In this manner, acetyl-CoA is increasingly made available not only for fatty acid biosynthesis but also for mevalonate metabolism (Fig. 1). The diversion of citrate for biosynthetic purposes bears the risk of collapsing the TCA cycle. To avoid this, activated T cells therefore increasingly use glutamine oxidation and glycolysis. Glutaminolysis represents a metabolic shunt that converts glutamine into α -ketoglutarate (α -KG) for subsequent introduction into the TCA cycle. In addition, reductive carboxylation of α -KG (the reverse of what occurs in the TCA cycle) for the subsequent synthesis of citrate and acetyl-CoA in the cytoplasm of activated T cells may also serve to fuel mevalonate metabolism, preferentially under hypoxic conditions (Fig. 1) (11).

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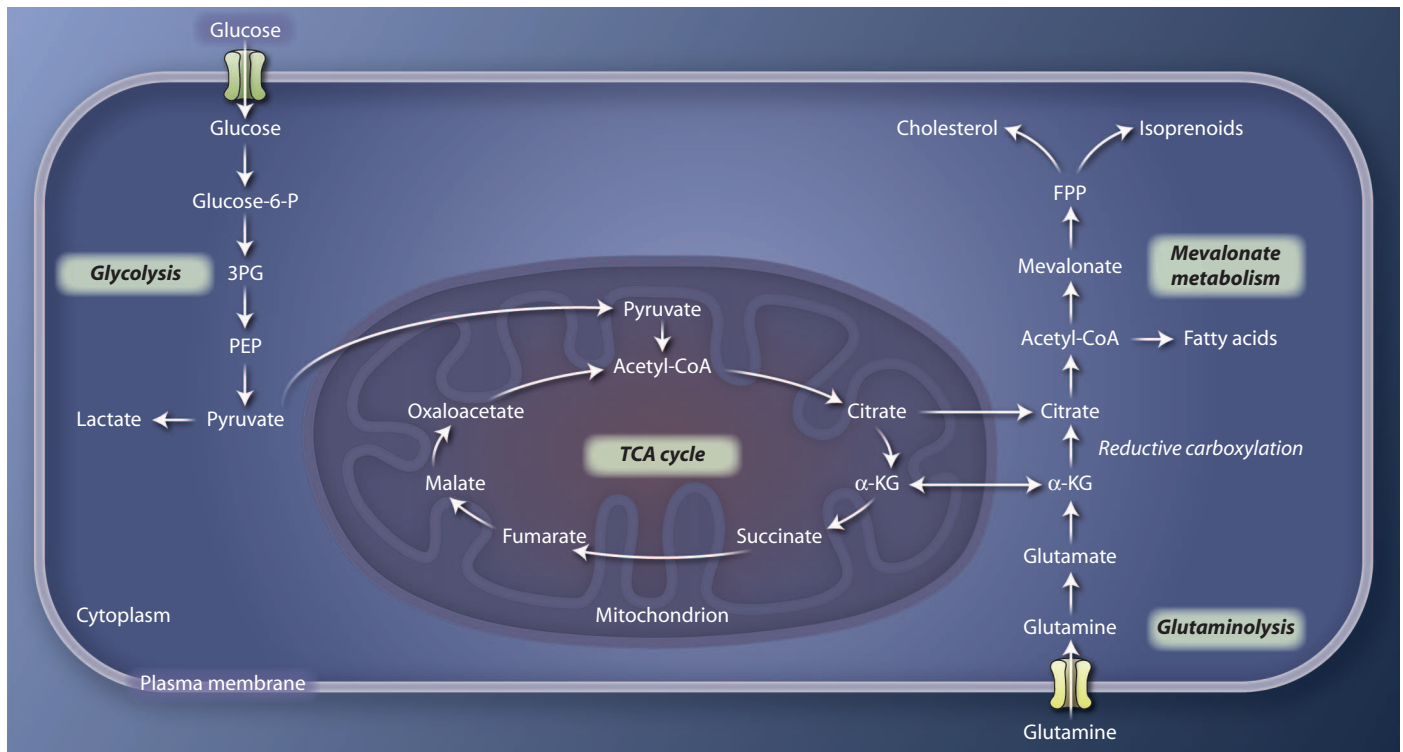


Fig. 1. Metabolic pathways that support T cell activation. In resting cells, glucose-derived pyruvate is converted to acetyl-CoA in mitochondria, and it is fully oxidized in the TCA cycle and through OXPHOS to maximize ATP generation. In contrast, proliferating T cells enhance glycolysis but also export citrate to the cytosol, where it is converted back to acetyl-CoA and serves as a metabolic precursor for fatty acid biosynthesis and mevalonate metabolism. To ensure metabolic flux, activated T cells also increase the amount of both glutamine oxidation and glycolysis. The

process of glutaminolysis represents a metabolic shunt that converts glutamine into α -KG for subsequent introduction into the TCA cycle. In addition, reductive carboxylation of α -KG (the reverse of what occurs to α -KG in the TCA cycle) for the subsequent synthesis of citrate and acetyl-CoA may also occur in the cytoplasm of activated T cells, preferentially in microenvironments with low oxygen, such as secondary lymphoid organs, inflamed tissues, and tumors. Glucose-6-P, glucose-6-phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate.

Metabolic Reprogramming During T Cell Activation Enhances Mevalonate Metabolism

Three molecules of acetyl-CoA can be sequentially condensed by 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase to form HMG-CoA, which enables the onset of mevalonate metabolism, a highly conserved anabolic cascade (Fig. 2). In the first committed step, HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate (also known as mevalonic acid) (12). In murine T cells, TCR stimulation is sufficient to induce expression of the genes encoding HMG-CoA synthase and HMG-CoA reductase (13, 14). The induction of this lipogenic program occurs at submitogenic concentrations of the specific antigen gp100 and does not require costimulation (13). Two sequential phosphorylation reactions generate mevalonate monophosphate and mevalonate diphosphate (also known as mevalonate pyrophosphate), respectively. Mevalonate pyrophosphate is decarboxylated to form isopentenyl pyrophosphate (IPP, C₅), whereas an isomerase catalyzes the interconversion of IPP and its isomer, dimethylallyl pyrophosphate (DMAPP, C₅). Farnesyl pyrophosphate (FPP) synthase condenses DMAPP with one IPP molecule to form geranyl pyrophosphate (GPP, C₁₀), as well as GPP with a second IPP to form FPP (C₁₅). FPP is the common substrate for various biosynthetic branches leading to diverse classes of products such as cholesterol, steroids, ubiquinones, and prenylated proteins (Fig. 2).

Regulation of Intracellular Cholesterol Homeostasis Affects T Cell Responses

FPP serves as the precursor in sterol biosynthesis (Fig. 2). In the first committed step, squalene synthase catalyzes, in a nicotinamide dinucleotide phosphate (NADPH)-dependent manner, the condensation of two FPP molecules (15). Squalene epoxidase, which catalyzes the first oxygenation step in sterol biosynthesis, is considered to be one of the rate-limiting enzymes in this pathway. Expression of the gene encoding squalene epoxidase is also induced as part of the TCR-stimulated lipid biosynthesis program that promotes HMG-CoA synthesis and reduction (13). Squalene oxidation followed by cyclization leads to the formation of lanosterol, which is then further converted to cholesterol through a multistep process. Feedback inhibition by cholesterol and the isoprenoid intermediates of the mevalonate pathway controls HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis and the target of a class of drugs known as statins (Fig. 2) (12).

Cholesterol is an essential component of membranes, and it ensures their permeability and fluidity. In addition, cholesterol is important for the formation of lipid rafts, which are specialized microdomains in the plasma membrane used for the assembly of signaling molecules (16). The requirement for adequate cholesterol availability in cell proliferation is therefore obvious. The absolute requirement for cholesterol in cell cycle

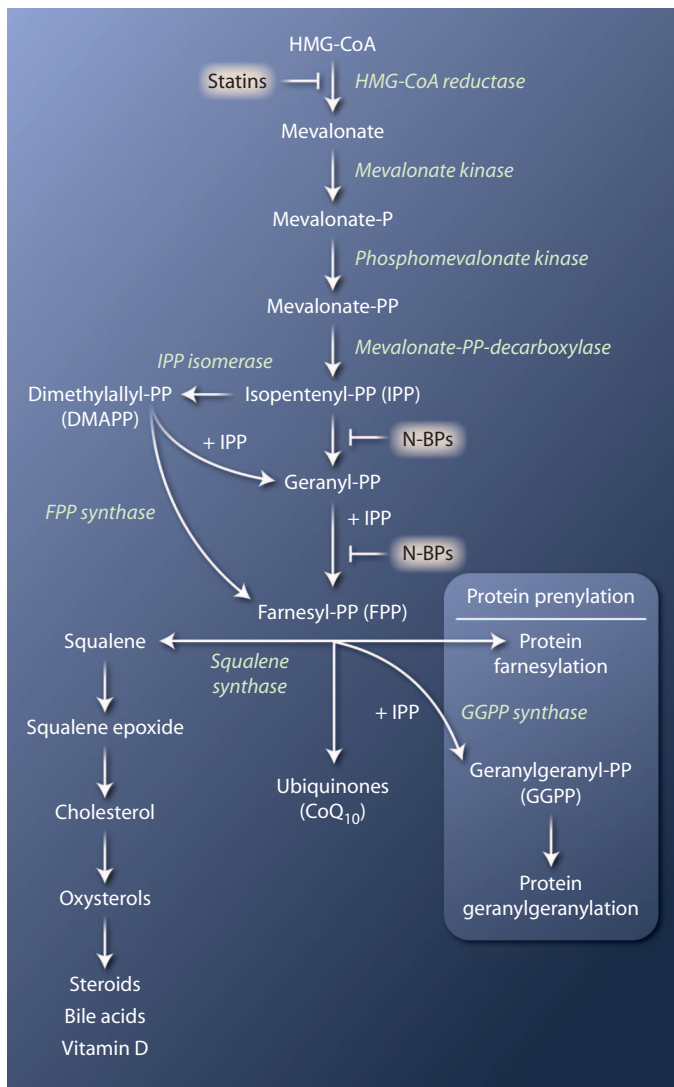


Fig. 2. Mevalonate metabolism. In the first committed step of the pathway, HMG-CoA reductase, the target of statins, catalyzes the formation of mevalonate. Two kinases and a decarboxylase then sequentially convert mevalonate into IPP, whereas an isomerase catalyzes the interconversion of IPP and its isomer, DMAPP. FPP synthase catalyzes sequential condensation reactions with DMAPP and two units of IPP to form FPP, which is the common substrate for the indicated biosynthetic branches. Finally, GGPP synthase catalyzes another condensation reaction with IPP to form GGPP. Farnesyltransferases and geranylgeranyltransferases use FPP and GGPP, respectively, as activated isoprenoid substrates in the posttranslational prenylation of proteins. By inhibiting FPP synthase, N-BPs cause the depletion of downstream FPP and GGPP (and thus inhibit protein prenylation) as well as the accumulation of IPP, a phosphoantigen that is specifically recognized by certain $\gamma\delta$ T lymphocytes (V γ δ 9V2 T cells). Mevalonate-P, mevalonate-5-phosphate.

progression and mitosis has previously been demonstrated with pharmacologic inhibitors of the mevalonate pathway, such as statins, which cause general cholesterol depletion (17, 18).

Physiologic regulation of sterol availability may be mediated by liver X receptors (LXRs), which are members of the nuclear receptor family of transcription factors (19). Accumulation of 22(R)-hydroxycholesterol (20), an oxysterol derivative of cholesterol (Fig. 2), results in the activation of LXR β in T cells and the suppression of mitogen- and antigen-dependent proliferation (21). LXR β is thought to promote the localized depletion of sterol by activating the cholesterol efflux transporter ATP-binding cassette subfamily G member 1 (ABCG1). Conversely, the absence of LXR β activity prevents the inhibitory effects of LXR β agonists and potentiates the homeostatic and vaccine-dependent proliferation of T cells (21). Similarly, genetic ablation of ABCG1 results in increased cholesterol content and confers thymocytes and CD4⁺ T cells with a proliferative advantage (22). Geranylgeranyl pyrophosphate (GGPP), which is produced in a nonsterol branch of the mevalonate pathway (Fig. 2), rapidly suppresses the cholesterol efflux transporter ABCA1 (23), which is indicative of collaborative interactions between different branches of the mevalonate pathway. In addition, oxysterols have been identified as agonist ligands of the retinoic acid receptor-related orphan receptors α and γ (ROR α and ROR γ t), which are members of the nuclear hormone receptor superfamily (24). Oxysterols are ROR γ t agonists and serve as potential endogenous ROR γ t ligands in promoting the differentiation of mouse and human CD4⁺ T helper 17 (T_H17) cells (25). Thus, LXR β acts as a sensor of intracellular sterol content and may couple sterol metabolism to innate and adaptive immune responses.

Another class of transcription factors that regulate sterol availability is constituted by the sterol regulatory element-binding proteins (SREBPs) (26). The 480-amino acid N-terminal residues of SREBPs form a basic helix-loop-helix leucine zipper transcription factor. In response to reduced amounts of sterols, this N-terminal domain of SREBPs is proteolytically released from the plasma membrane and is relocalized to the nucleus, where it binds to sterol regulatory elements and thus enhances the expression of genes encoding enzymes involved in fatty acid and sterol biosynthesis. Three SREBP isoforms have been identified. SREBP-1a potentially activates all SREBP-responsive genes and may function to maintain the basal synthesis of cholesterol and fatty acids. In contrast, SREBP-1c selectively activates genes whose products are involved in fatty acid synthesis, whereas SREBP-2 preferentially promotes mevalonate metabolism and cholesterol biosynthesis by activating the transcription of the genes encoding HMG-CoA synthase and HMG-CoA reductase. SREBP-1 and SREBP-2 are essential in coupling TCR signaling with a lipid biosynthetic program that ensures rapid membrane biogenesis and cell growth (13).

Murine CD8⁺ T cells defective in SREBP signaling appear to be unable to synthesize sufficient cholesterol for cell growth and proliferation in response to antigenic stimulation. SREBP signaling is dispensable for the homeostatic proliferation of CD8⁺ T cells, but it is required for CD8⁺ T cells to acquire the glycolytic phenotype that enables full effector function. Such a concept, in which an intracellular cholesterol pool serves as a metabolic checkpoint for the development of effector T cells, would also be consistent with the previous observation that cholesterol abundance is increased in $\gamma\delta$ T cells compared to that in $\alpha\beta$ T cells, and that this may contribute to the rapid, innate-like effector responses of $\gamma\delta$ T cells (27). Furthermore, this concept may even apply to regulatory T cells (T_{regs}), which also appear to require sufficient metabolic flux through the mevalonate pathway to become fully functional (28, 29).

Disruption of mechanistic target of rapamycin complex 1 (mTORC1) by genetic depletion of regulatory-associated protein of mTOR (Raptor) results in the decreased abundance of HMG-CoA reductase, the enzyme that catalyzes the first committed step of the mevalonate pathway, as well as of squalene epoxidase, one of the rate-limiting enzymes in cholesterol biosynthesis. As a consequence, Raptor-deficient T_{regs} have reduced amounts

of cellular cholesterol, as well as decreased glycolytic activity, compared to wild-type T_{regs} , which suggests that effector T cell function and T_{reg} competence may have similar metabolic requirements. Inhibiting mevalonate metabolism in wild-type T_{regs} with statins, which inhibit HMG-CoA reductase, likewise blocks their suppressive activity, an effect that is completely reversed by the addition of mevalonate, the metabolite downstream of HMG-CoA reductase (28).

T Cell Regulation by Steroid Hormones

Oxysterols can serve as precursors in the biosynthesis of steroid hormones (Fig. 2) (20), which have various immunomodulatory effects. Whereas estrogens are considered to enhance antibody production in response to vaccines, as well as in autoimmune diseases in women (30), androgens may suppress immune responses and play a critical role in maintaining T cell tolerance. For example, castration of male mice before their vaccination with prostate-specific antigen enhances antigen-specific $CD8^+$ T cell responses (31, 32). Likewise, prostate cancer patients undergoing androgen deprivation have increased infiltration of the prostate with T cells compared to prostate cancer patients that do not receive hormone ablation therapy (33). A study pinpointed testosterone-mediated inhibition of T_H1 cell differentiation as the molecular mechanism by which androgens suppress immunity. Testosterone inhibits the interleukin-12 (IL-12)-dependent phosphorylation of signal transducer and activator of transcription 4 in $CD4^+$ T cells, an effect that is mediated by the binding of the androgen receptor to a conserved region of the protein tyrosine phosphatase non-receptor type 1 (Ptpn1), which is followed by an increase in Ptpn1 expression and activity as well as by the subsequent suppression of IL-12 signaling (34).

Gender-specific differences in phenotype and function have also been reported for human $V\gamma9V\delta2$ T cells (35). This population of unconventional, innate-like, $\gamma\delta$ T cells is well known to increase in number from birth to puberty and then gradually declines beyond 30 years of age. The postpuberty decrease in cell number is more pronounced in men, whereas women retain higher numbers of $V\gamma9V\delta2$ T cells. The loss of $V\gamma9V\delta2$ T cells in men appears to specifically affect effector memory and terminally differentiated effector T cells, whereas the population of central memory cells is unaffected. Although the underlying molecular mechanisms are currently unknown, it is likely that steroid hormones and sterol metabolism contribute to the observed gender differences in the differentiation of $V\gamma9V\delta2$ T cells.

Mevalonate Metabolism Is Required for OXPHOS in T Cells to Ensure Ubiquinone Availability

Ubiquinone [also known as coenzyme Q (CoQ)] (Fig. 2) plays a unique role in the electron transfer chain, where it functions as an electron carrier from complex I and complex II to complex III. CoQ-cytochrome c reductase is a component of the ubiquinol-cytochrome c reductase complex (complex III or cytochrome bc1 complex), the third complex in the electron transport chain, and it plays a critical role in ATP synthesis during OXPHOS. This enzyme catalyzes the reduction of cytochrome c by oxidation of CoQ and the concomitant pumping of protons from the mitochondrial matrix to the intermembrane space. The net result of this redox loop, which is also referred to as the Q cycle, is that two electrons are transferred from a molecule of reduced CoQ (ubiquinol) to two molecules of oxidized cytochrome c. This transfer results in the formation of two molecules of reduced cytochrome c and one molecule of oxidized CoQ (ubiquinone).

Indirect evidence for the importance of CoQ in antigen-specific T cell activation comes from a study showing that mice deficient in CoQ-cytochrome c

reductase fail to exhibit antigen-specific proliferation of T cells in vivo. This is because intact mitochondria are required for T cell activation to produce reactive oxygen species (ROS) for the activation of nuclear factor of activated T cells and the subsequent induction of *IL2* expression (36). Mitochondrial glycerol-3-phosphate dehydrogenase (GPD2) is a major contributor of electrons to the respiratory chain. The TCR-dependent activation of GPD2 results in the enhanced reduction of ubiquinone (ubiquinol accumulation) and the release of low amounts of ROS from the mitochondria (37). The resulting oxidative signal is indispensable for T cell activation and for the nuclear factor κ B-dependent expression of target genes.

Little is known about ubiquinone biosynthesis in humans. Prenyldiphosphate synthase catalyzes the elongation of GPP or FPP with several IPP moieties in a trans configuration to form the polyisoprenoid chain. A *trans*-polyprenyl transferase then attaches the polyisoprenyl side chain to a 4-hydroxybenzoate ring, thus producing a membrane-bound ubiquinone. In CoQ₁₀, which plays a pivotal role in OXPHOS, the lipid membrane anchor is a decaprenyl side chain (10 isoprenyl units, C₅₀). Because of the relatively large number of isoprenyl units required for CoQ₁₀ biosynthesis, CoQ₁₀ availability obviously depends on high flux through the mevalonate pathway, and conversely, inefficient mevalonate metabolism would rapidly lead to the depletion of CoQ₁₀ and the subsequent collapse of OXPHOS. Clinically, mutations in the genes encoding either prenyldiphosphate synthase or *trans*-polyprenyl transferase result in CoQ₁₀ deficiency and OXPHOS disorders (38). In addition to such inborn errors of CoQ₁₀ biosynthesis, drugs targeting the mevalonate pathway may also cause CoQ₁₀ deficiency. Statins, which are widely prescribed for the treatment of cardiovascular disease, inhibit mevalonate metabolism at an early stage (Fig. 2) and thus also prevent the formation of the ubiquinone lipid anchor. Statins can therefore cause CoQ₁₀ deficiency in a dose-dependent manner, and they may have detrimental cardiac consequences, mostly in elderly patients (39). These observations collectively suggest that mevalonate metabolism controls OXPHOS in various cell types, including T cells.

Protein Prenylation Determines T Cell Function

The farnesyl and geranylgeranyl transferases mediate the posttranslational prenylation of proteins (15). In this process, FPP (C₁₅) and GGPP (C₂₀) respectively represent the activated forms of the farnesyl and geranylgeranyl units that are covalently attached to many members of the Ras superfamily of guanosine triphosphatases (GTPases), which mediate their membrane localization (Fig. 2) (40). Notably, prenylated proteins may constitute up to 2% of total cellular protein (41). The prototype of small GTPases, Ras, which requires farnesylation (FPP), is activated in response to TCR stimulation and various cytokines (Fig. 3A) (42). The best-characterized effectors of Ras are the Raf proteins, which are kinases (43) that control T cell proliferation, cytokine production, differentiation, and survival through activation of the mitogen-activated protein kinase (MAPK) cascade (Fig. 3A). Consistently, statin-dependent inhibition of the farnesylation of Ras impairs the association of Ras with lipid rafts and prevents the TCR-dependent activation of the Ras-MAPK pathway (44).

The role of Ras farnesylation in the differentiation of T_H cells has also been investigated in the context of the experimental autoimmune encephalomyelitis (EAE) rodent model of multiple sclerosis (45). This study showed that statins induce a decrease in the amount of membrane-associated Ras as well as in the concomitant accumulation of cytosolic Ras, and they inhibit the destructive T_H1 cell-type response. FPP, which mediates Ras farnesylation, restores the ability of statin-treated, myelin-reactive T cells to produce the effector cytokine interferon- γ (IFN- γ).

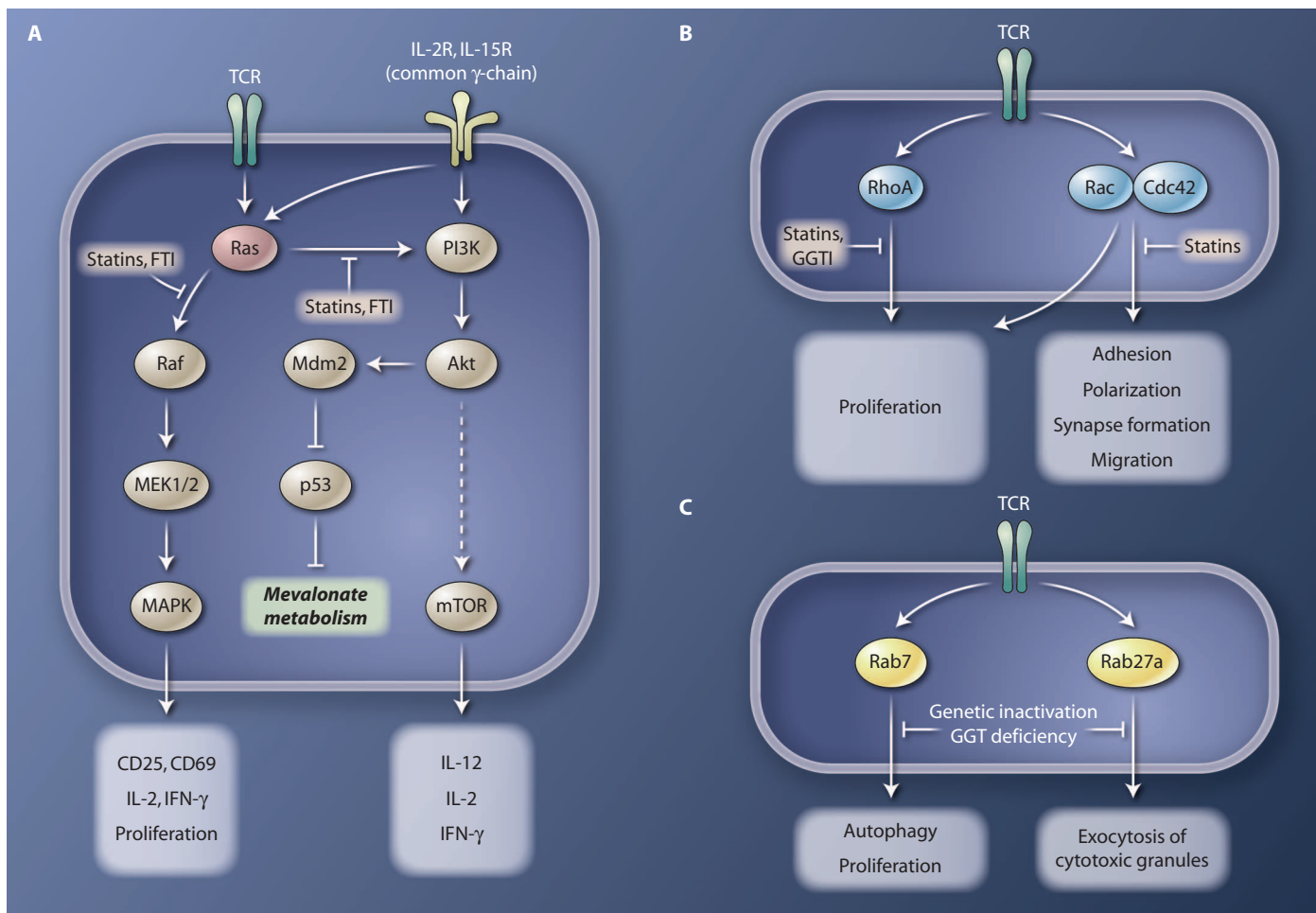


Fig. 3. Prenylated proteins in T cell activation. (A) The two best-studied effector pathways that are activated by RAS in response to the stimulation of T cells through the TCR or by cytokines are the RAF-MEK-MAPK signaling cascade and the PI3K-AKT-mTOR pathway. AKT can also phosphorylate cellular proteins in a process that results in the inhibition of p53. This AKT-dependent pathway might also abolish the suppressive effect that p53 has on mevalonate metabolism. Because Ras depends on farnesylation to become active, statins, which inhibit mevalonate metabolism at an early stage, or specific farnesyltransferase inhibitors (FTI) block these effector pathways and thus inhibit T cell

activation, proliferation, and cytokine production. (B) The TCR-stimulated activation of RhoA and Rac2 results in T cell proliferation, whereas the activation of Rac1 and Cdc42 is required for cytoskeletal dynamics. Inhibition of geranylgeranylation by statins or specific geranylgeranyltransferase inhibitors (GGTIs) results in the inactivation of Rho GTPases and the abrogation of T cell responses. (C) Membrane trafficking of vesicles elicited by T cell activation involves Rab proteins, which are often doubly geranylgeranylated. Genetic inactivation of Rab proteins or of geranylgeranyltransferase (GGT) prevents proper T cell activation and effector function. IL-2R, IL-2 receptor.

Reinstatement of metabolism by the provision of exogenous FPP during statin-mediated inhibition of HMG-CoA reductase, and thus of mevalonate metabolism, indicates that mevalonate metabolites can also act as cell-extrinsic cues. The involvement of farnesylated Ras in the differentiation of T_H1 cells was also confirmed in experiments with a specific farnesyltransferase inhibitor.

Ras activates not only the MAPK signaling cascade but also the phosphoinositide 3-kinase (PI3K)-AKT pathway (46, 47), which is also activated during IL-2- and IL-15-induced effector T cell responses (Fig. 3A) (48, 49). The importance of Ras farnesylation in this pathway was demonstrated by the finding that specific farnesyltransferase inhibitors suppress PI3K-AKT signaling (46). AKT phosphorylates multiple cellular proteins, which results in the inhibition of several tumor suppressors, including p53, which normally inhibits mevalonate metabolism. A reduction in the abun-

dance of p53 by the specific ubiquitin ligase Mdm2 (Fig. 3A) has been demonstrated during T cell activation (50), which implies that mevalonate metabolism may be enhanced in tumor cells and in activated T cells by similar signaling events. The activity of the mevalonate pathway in breast cancer cells bearing mutant p53 is substantially enhanced compared to that in breast cancer cells expressing wild-type p53 (51). Thus, Ras, which depends on mevalonate-derived FPP to be farnesylated, may also enhance mevalonate metabolism by suppressing p53, which is indicative of the formation of a feedforward loop that enhances growth-promoting cellular metabolism (Fig. 3A). Similarly, the IL-2- or IL-15-dependent activation of PI3K-AKT signaling may also stimulate mevalonate pathway activity.

The Rho GTPases RhoA, Cdc42, and Rac play key roles in a wide range of biological processes (52, 53). To become functionally active, these GTPases must undergo geranylgeranylation. Early work demonstrated that

RhoA is a positive regulator of TCR responses (Fig. 3B) (54). T cells of transgenic mice expressing an active mutant of RhoA show enhanced responses in the context of TCR-induced proliferation *in vitro* and *in vivo*. In the EAE rodent model, statins decrease the amount of membrane-associated RhoA and inhibit the proliferation of myelin-reactive T cells. GGPP, the lipid moiety required for the geranylgeranylation of RhoA, reverses the inhibition of T cell proliferation caused by statins (45). The involvement of geranylgeranylated RhoA in T cell proliferation was also confirmed in experiments with a specific geranylgeranyltransferase inhibitor.

The GTPases Rac and Cdc42, which can be activated by Zap70 (55), a key element in TCR signaling, are pivotal regulators of cytoskeletal movement in response to the antigen-specific activation of T cells by antigen-presenting cells (APCs) (56). Early work showed that Cdc42 mediates T cell polarization toward APCs (Fig. 3B) (57). The Rho GTPase-dependent reorganization of the cytoskeleton is also a critical process in driving receptor clustering, leading to synapse formation (Fig. 3B) (56). In mice lacking Rac2, a hematopoietic cell-specific isoform of Rac, actin reorganization and polymerization in T cells is impaired, which results in their reduced proliferative capacity (58). These results indicate that Rac2 is important in mediating both proliferation and cytoskeletal changes during T cell activation (Fig. 3B). Statins interfere with the association of Rac with the plasma membrane by inhibiting its prenylation and, as a consequence,

preventing the TCR-dependent activation of the stress-responsive MAPK p38 (44).

Rab proteins, which are modified by the attachment of geranylgeranyl groups (usually two) to the C terminus of the protein (59), are involved in almost every route of intracellular trafficking (60). Autophagy is a conserved degradation process and has been implicated in T cell proliferation and effector function. T cells from mice with a T cell-specific deficiency in Rab7 exhibit a profound block in autophagosome degradation (61). Such Rab7-deficient T cells are sensitive to limiting amounts of extracellular nutrients and exhibit defects in proliferation after TCR stimulation (Fig. 3C) (61). Because most Rab proteins are posttranslationally modified with two geranylgeranyl lipid moieties, they are particularly sensitive to the effects of mevalonate pathway inhibitors such as statins. Statins cause considerable cell stress and induce stress responses, including the unfolded protein response (UPR) and autophagy (62). Because autophagy depends on Rab proteins, autophagy cannot be completed during statin-mediated inhibition of prenylation, and it is therefore abandoned. A well-established effect of blocking autophagy is the increased ROS production from mitochondria that fail to be cleared by mitophagy (63). ROS, in turn, can activate inflammasomes (64, 65). Consistent with this, treatment with statins or nitrogen-containing bisphosphonates (N-BPs) induces inflammasome and caspase-1 activation, which leads to the production of the

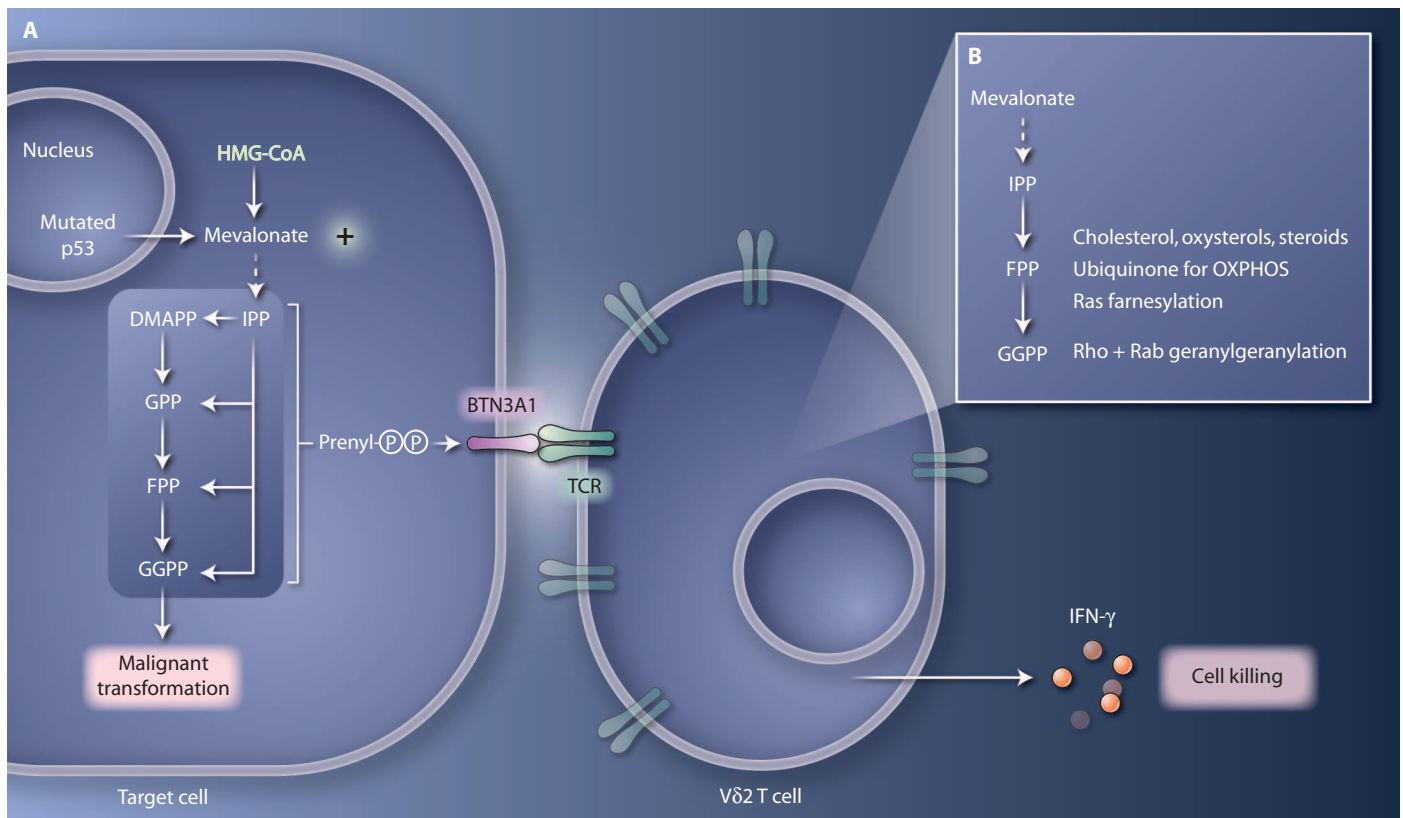


Fig. 4. Regulation of $\gamma\delta$ T cells by mevalonate metabolites: A special case. In the case of V γ 9V δ 2 T cells, the mevalonate pathway provides not only metabolites but also multiple TCR agonists (IPP, DMAPP, GPP, FPP, and GGPP). These mevalonate metabolites may thus play a dual role as cell-extrinsic and cell-intrinsic cues. (A) When mevalonate metabolites accumulate in target cells, for example, in tumor cells that bear a mutated p53 protein and thus have an enhanced mevalonate metabolism, they may bind to BTN3A1 and

mediate its translocation to the plasma membrane, which leads to the engagement of the V γ 9V δ 2 TCR on V δ 2 T cells. Stimulation of this TCR leads to rapid, innate cell-like effector functions, including IFN- γ production and target cell killing. (B) In addition, cell-intrinsic FPP and GGPP in V γ 9V δ 2 T cells may serve as isoprenoid precursor units in the biosynthesis of cholesterol, oxysterols, steroids, ubiquinone, and prenylated proteins, which thus contribute to the regulation of V γ 9V δ 2 T cell function at a range of different levels.

proinflammatory cytokines IL-1 β and IL-18 (66), which contribute to the activation of $\gamma\delta$ T cells preactivated with IL-2 (67).

Members of the Rab family of GTPases, such as Rab27a, also regulate discrete steps in granule exocytosis from cytotoxic T cells (Fig. 3C). Resting T lymphocytes do not contain cytotoxic granules; their biogenesis requires T cell activation. Rab27a is required in a final secretory step of granule polarization toward the immunological synapse (68–70). In *gunmetal*, a murine model of Hermansky-Pudlak syndrome (71), Rab27a dysfunction is secondary to a mutation in the α subunit of Rab geranylgeranyltransferase, the enzyme required for the prenylation and activation of Rab proteins (Fig. 3C) (72). In cytotoxic T cells from *gunmetal* mice, insufficient prenylation of Rab27a leads to a failure in granule polarization and, thus, in the reduced ability of these cytotoxic T cells to kill target cells (70). Not only is Rab27 required for effector T cell function, but it also may contribute to the suppressive activity of T_{regs}. A study showed that T_{regs} transfer gene-silencing microRNAs through exosomes to various immune cells, including T_H1 cells, which suppress cellular proliferation and cytokine secretion (73). This form of T_{reg}-mediated suppression depends on Rab27 because the release of exosomes requires Rab27a and Rab27b to dock multivesicular endosomes to Rab27 effectors on the plasma membrane (74).

Multiple Mevalonate Metabolites Are Potent Agonists of $\gamma\delta$ T Cells: A Special Case

With regard to V γ 9V δ 2 T cells, which are primate-specific $\gamma\delta$ T cells and are not present in mice, the mevalonate pathway is distinct because it provides not only metabolic intermediates but also the cognate antigen IPP (75). IPP, which is one of the oldest known biomolecules (15), is also produced by bacteria, including mycobacteria, protozoa, and parasites through the non-mevalonate pathway of isoprenoid biosynthesis. V γ 9V δ 2 T cells can thus contribute to the surveillance of metabolic stress and pathogens. Dysregulation of the mevalonate pathway activates V γ 9V δ 2 T cells. For example, N-BPs, such as zoledronate, which represents the standard of care in the treatment of bone disease in patients with multiple myeloma (76), inhibit FPP synthase (Fig. 2) (12). The resulting depletion of FPP and of downstream GGPP, which prevents protein farnesylation and geranylgeranylation, respectively, prevents bone degradation and exerts direct and indirect antitumor effects (15, 72, 77). An on-target side effect of N-BPs, however, is the upstream accumulation of IPP, which activates V γ 9V δ 2 T cells (75, 78–81). IPP, which is characterized as a mycobacterial antigen that stimulates $\gamma\delta$ T cells (82), is therefore considered to be the mevalonate metabolite that is responsible for the N-BP-dependent activation of $\gamma\delta$ T cells.

The observation that N-BPs, such as zoledronate, activate $\gamma\delta$ T cells despite the depletion of downstream metabolites suggests that these compounds may be less relevant (or even irrelevant) as $\gamma\delta$ T cell agonists; however, a study has challenged this view. V γ 9V δ 2 T cells are potently activated not only by IPP but also by the downstream isoprenoid pyrophosphates DMAPP, GPP, FPP, and GGPP (83). Moreover, pretreatment with the stress-related, inflammasome-dependent cytokine IL-18 enhances the responses of V γ 9V δ 2 T cells to all mevalonate-derived isoprenoid pyrophosphates. These findings suggest that the mevalonate pathway provides multiple potent $\gamma\delta$ T cell agonists and that $\gamma\delta$ T cells may perform comprehensive surveillance of mevalonate metabolism, which may indeed be required because enhanced flux through the growth-promoting mevalonate pathway can lead to malignant transformation (Fig. 4A) (84, 85).

The ubiquitous protein butyrophilin 3A1 (BTN3A1, also known as CD277) binds to phosphorylated antigens, such as IPP, and activates V γ 9V δ 2 T cells (86, 87). However, in contrast to a typical antigen-presenting molecule, BTN3A1 binds to phosphorylated antigens with its intracellular B30.2 domain through a positively charged surface pocket (88), which suggests

that BTN3A1 may act as a sensor of the intracellular abundance of such phosphorylated antigens. When intracellular phosphorylated antigens reach a critical amount, for example, in a tumor cell with a hyperactive mevalonate metabolism, they appear to mediate the cell surface immobilization of BTN3A1, which leads to engagement of the V γ 9V δ 2 TCR and T cell activation (Fig. 4A). These findings also raise the question of whether such mevalonate-derived phosphorylated compounds should be referred to as antigens. They might more likely be considered as metabolic cues that act as danger signals. In addition to being agonists of V γ 9V δ 2 T cells, $\gamma\delta$ T cell–intrinsic mevalonate metabolites may contribute to the regulation of V γ 9V δ 2 T cell fitness and function (Fig. 4B).

Concluding Remarks

It is now clear that the activation and proliferation of T cells requires enhanced metabolic flux through growth-promoting pathways. Activated T cells take advantage of the Warburg effect to rapidly process glucose and to funnel acetyl-CoA into mevalonate metabolism. T cells thus represent an attractive means to examine how the usage of distinct metabolic pathways translates into cellular proliferation or effector responses. Studies have generated evidence that mevalonate metabolites regulate conventional and nonconventional T cells at a range of different levels, but the elucidation of the exact mechanisms of action of mevalonate-derived intermediates in various T cell functions still requires substantial work. One limitation lies in the requirement for specialized methodology and instrumentation to study metabolism. The acquisition of sufficient cellular material represents yet another challenge, particularly when primary T cells that occur at low frequencies, such as $\gamma\delta$ T cells, are examined. However, once these technical barriers are overcome, an improved understanding of mevalonate metabolism will enable more effective T cell manipulation for immunotherapeutic purposes in cancer and other diseases.

REFERENCES AND NOTES

1. B. Alarcon, H. M. van Santen, Two receptors, two kinases, and T cell lineage determination. *Sci. Signal.* **3**, pe11 (2010).
2. N. J. MacIver, R. D. Michalek, J. C. Rathmell, Metabolic regulation of T lymphocytes. *Annu. Rev. Immunol.* **31**, 259–283 (2013).
3. E. L. Pearce, M. C. Poffenberger, C. H. Chang, R. G. Jones, Fueling immunity: Insights into metabolism and lymphocyte function. *Science* **342**, 1242454 (2013).
4. R. Wang, D. R. Green, Metabolic checkpoints in activated T cells. *Nat. Immunol.* **13**, 907–915 (2012).
5. E. L. Pearce, E. J. Pearce, Metabolic pathways in immune cell activation and quiescence. *Immunity* **38**, 633–643 (2013).
6. R. Wang, C. P. Dillon, L. Z. Shi, S. Milasta, R. Carter, D. Finkelstein, L. L. McCormick, P. Fitzgerald, H. Chi, J. Munger, D. R. Green, The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* **35**, 871–882 (2011).
7. R. D. Michalek, V. A. Gerriets, A. G. Nichols, M. Inoue, D. Kazmin, C. Y. Chang, M. A. Dwyer, E. R. Nelson, K. N. Pollizzi, O. Ilkayeva, V. Giguere, W. J. Zuercher, J. D. Powell, M. L. Shinohara, D. P. McDonnell, J. C. Rathmell, Estrogen-related receptor- α is a metabolic regulator of effector T-cell activation and differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 18348–18353 (2011).
8. C. H. Chang, J. D. Curtis, L. B. Maggi Jr., B. Faubert, A. V. Villarino, D. O'Sullivan, S. C. Huang, G. J. van der Windt, J. Blagih, J. Qiu, J. D. Weber, E. J. Pearce, R. G. Jones, E. L. Pearce, Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* **153**, 1239–1251 (2013).
9. R. M. Laird, B. J. Wolf, M. F. Princiotta, S. M. Hayes, $\gamma\delta$ T cells acquire effector fates in the thymus and differentiate into cytokine-producing effectors in a *Listeria* model of infection independently of CD28 costimulation. *PLoS One* **8**, e63178 (2013).
10. G. Kroemer, J. Pouyssegur, Tumor cell metabolism: Cancer's Achilles' heel. *Cancer Cell* **153**, 1239–1251 (2008).
11. C. M. Metallo, P. A. Gameiro, E. L. Bell, K. R. Mattaini, J. Yang, K. Hiller, C. M. Jewell, Z. R. Johnson, D. J. Irvine, L. Guarente, J. K. Kelleher, M. G. Vander Heiden, O. Iliopoulos, G. Stephanopoulos, Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* **481**, 380–384 (2011).
12. J. L. Goldstein, M. S. Brown, Regulation of the mevalonate pathway. *Nature* **343**, 425–430 (1990).

13. Y. Kidani, H. Elsaesser, M. B. Hock, L. Vergnes, K. J. Williams, J. P. Argus, B. N. Marbois, E. Komisopoulou, E. B. Wilson, T. F. Osborne, T. G. Graeber, K. Reue, D. G. Brooks, S. J. Bensinger, Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat. Immunol.* **14**, 489–499 (2013).
14. R. Chakrabarti, E. G. Engleman, Interrelationships between mevalonate metabolism and the mitogenic signaling pathway in T lymphocyte proliferation. *J. Biol. Chem.* **266**, 12216–12222 (1991).
15. M. Thurnher, G. Gruenbacher, O. Nussbaumer, Regulation of mevalonate metabolism in cancer and immune cells. *Biochim. Biophys. Acta* **1831**, 1009–1015 (2013).
16. D. Lingwood, K. Simons, Lipid rafts as a membrane-organizing principle. *Science* **327**, 46–50 (2010).
17. J. Martínez-Botas, A. J. Ferruelo, Y. Suarez, C. Fernandez, D. Gomez-Coronado, M. A. Lasuncion, Dose-dependent effects of lovastatin on cell cycle progression. Distinct requirement of cholesterol and non-sterol mevalonate derivatives. *Biochim. Biophys. Acta* **1532**, 185–194 (2001).
18. J. Martínez-Botas, Y. Suárez, A. J. Ferruelo, D. Gómez-Coronado, M. A. Lasunción, Cholesterol starvation decreases p34^{cdc2} kinase activity and arrests the cell cycle at G2. *FASEB J.* **13**, 1359–1370 (1999).
19. Y. Kidani, S. J. Bensinger, Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. *Immunol. Rev.* **249**, 72–83 (2012).
20. B. A. Janowski, P. J. Willy, T. R. Devi, J. R. Falck, D. J. Mangelsdorf, An oxysterol signalling pathway mediated by the nuclear receptor LXR α . *Nature* **383**, 728–731 (1996).
21. S. J. Bensinger, M. N. Bradley, S. B. Joseph, N. Zelcer, E. M. Janssen, M. A. Hausner, R. Shih, J. S. Parks, P. A. Edwards, B. D. Jamieson, P. Tontonoz, LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell* **134**, 97–111 (2008).
22. A. J. Armstrong, A. K. Gebre, J. S. Parks, C. C. Hedrick, ATP-binding cassette transporter G1 negatively regulates thymocyte and peripheral lymphocyte proliferation. *J. Immunol.* **184**, 173–183 (2010).
23. X. Gan, R. Kaplan, J. G. Menke, K. MacNaul, Y. Chen, C. P. Sparrow, G. Zhou, S. D. Wright, T. Q. Cai, Dual mechanisms of ABCA1 regulation by geranylgeranyl pyrophosphate. *J. Biol. Chem.* **276**, 48702–48708 (2001).
24. Y. Wang, N. Kumar, C. Crumbley, P. R. Griffin, T. P. Burris, A second class of nuclear receptors for oxysterols: Regulation of ROR α and ROR γ activity by 24S-hydroxycholesterol (cerebrosterol). *Biochim. Biophys. Acta* **1801**, 917–923 (2010).
25. P. Sorosh, J. Wu, X. Xue, J. Song, S. W. Sutton, M. Sablad, J. Yu, M. I. Nelen, X. Liu, G. Castro, R. Luna, S. Crawford, H. Banie, R. A. Dandridge, X. Deng, A. Bittner, C. Kuei, M. Tootoonchi, N. Rozenkrants, K. Herman, J. Gao, X. V. Yang, K. Sachen, K. Ngo, W. P. Fung-Leung, S. Nguyen, A. de Leon-Tabaldo, J. Blevitt, Y. Zhang, M. D. Cummings, T. Rao, N. S. Mani, C. Liu, M. McKinnon, M. E. Milla, A. M. Fourie, S. Sun, Oxysterols are agonist ligands of ROR γ t and drive Th17 cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12163–12168 (2014).
26. M. S. Brown, J. L. Goldstein, The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**, 331–340 (1997).
27. H. Y. Cheng, R. Wu, A. K. Gebre, R. N. Hanna, D. J. Smith, J. S. Parks, K. Ley, C. C. Hedrick, Increased cholesterol content in gamma delta ($\gamma\delta$) T lymphocytes differentially regulates their activation. *PLOS One* **8**, e63746 (2013).
28. H. Zeng, K. Yang, C. Cloer, G. Neale, P. Vogel, H. Chi, mTORC1 couples immune signals and metabolic programming to establish T_{reg}-cell function. *Nature* **499**, 485–490 (2013).
29. H. Zeng, H. Chi, The interplay between regulatory T cells and metabolism in immune regulation. *Oncoimmunology* **2**, e26586 (2013).
30. S. L. Klein, A. Jedlicka, A. Pekosz, The Xs and Y of immune responses to viral vaccines. *Lancet Infect. Dis.* **10**, 338–349 (2010).
31. M. S. Arredouani, S. S. Tseng-Rogenski, B. K. Hollenbeck, J. Escara-Wilke, K. R. Leander, D. Defeo-Jones, C. Hwang, M. G. Sanda, Androgen ablation augments human HLA2.1-restricted T cell responses to PSA self-antigen in transgenic mice. *Prostate* **70**, 1002–1011 (2010).
32. A. C. Roden, M. T. Moser, S. D. Tri, M. Mercader, S. M. Kuntz, H. Dong, A. A. Hurwitz, D. J. McKean, E. Celis, B. C. Leibovich, J. P. Allison, E. D. Kwon, Augmentation of T cell levels and responses induced by androgen deprivation. *J. Immunol.* **173**, 6098–6108 (2004).
33. M. Mercader, B. K. Bodner, M. T. Moser, P. S. Kwon, E. S. Park, R. G. Manecke, T. M. Ellis, E. M. Wojcik, D. Yang, R. C. Flanagan, W. B. Waters, W. M. Kast, E. D. Kwon, T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14565–14570 (2001).
34. H. T. Kissick, M. G. Sanda, L. K. Dunn, K. L. Pellegrini, S. T. On, J. K. Noel, M. S. Arredouani, Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 9887–9892 (2014).
35. N. Caccamo, F. Dieli, D. Wesch, H. Jomaa, M. Eberl, Sex-specific phenotypical and functional differences in peripheral human V γ 9/V δ 2 T cells. *J. Leukoc. Biol.* **79**, 663–666 (2006).
36. L. A. Sena, S. Li, A. Jairaman, M. Prakriya, T. Ezponda, D. A. Hildeman, C. R. Wang, P. T. Schumacker, J. D. Licht, H. Perlman, P. J. Bryce, N. S. Chandel, Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* **38**, 225–236 (2013).
37. M. M. Kamiński, S. W. Sauer, M. Kamiński, S. Opp, T. Ruppert, P. Grigaričius, P. Grudnik, H. J. Gröne, P. H. Krammer, K. Gülow, T cell activation is driven by an ADP-dependent glucokinase linking enhanced glycolysis with mitochondrial reactive oxygen species generation. *Cell Rep.* **2**, 1300–1315 (2012).
38. J. Mollet, I. Giurgea, D. Schlemmer, G. Dallner, D. Chretien, A. Delahodde, D. Bacq, P. de Lonlay, A. Munnich, A. Rötig, *Prenyl(diphosphate synthase, subunit 1 (PDS1)* and *OH-benzoate polyprenyltransferase (COQ2)* mutations in ubiquinone deficiency and oxidative phosphorylation disorders. *J. Clin. Invest.* **117**, 765–772 (2007).
39. C. R. Harper, T. A. Jacobson, The broad spectrum of statin myopathy: From myalgia to rhabdomyolysis. *Curr. Opin. Lipidol.* **18**, 401–408 (2007).
40. K. Wennerberg, K. L. Rossman, C. J. Der, The Ras superfamily at a glance. *J. Cell Sci.* **118**, 843–846 (2005).
41. J. Greenwood, L. Steinman, S. S. Zamvil, Statin therapy and autoimmune disease: From protein prenylation to immunomodulation. *Nat. Rev. Immunol.* **6**, 358–370 (2006).
42. D. A. Cantrell, GTPases and T cell activation. *Immunol. Rev.* **192**, 122–130 (2003).
43. E. Ligeti, S. Welts, K. Scheffzek, Inhibition and termination of physiological responses by GTPase activating proteins. *Physiol. Rev.* **92**, 237–272 (2012).
44. R. Ghittoni, L. Patrussi, K. Pirozzi, M. Pellegrini, P. E. Lazzarini, P. L. Capecchi, F. L. Pasini, C. T. Baldari, Simvastatin inhibits T-cell activation by selectively impairing the function of Ras superfamily GTPases. *FASEB J.* **19**, 605–607 (2005).
45. S. E. Dunn, S. Youssef, M. J. Goldstein, T. Prod'homme, M. S. Weber, S. S. Zamvil, L. Steinman, Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. *J. Exp. Med.* **203**, 401–412 (2006).
46. N. Berndt, A. D. Hamilton, S. M. Sebti, Targeting protein prenylation for cancer therapy. *Nat. Rev. Cancer* **11**, 775–791 (2011).
47. K. Okkenhaug, Signaling by the phosphoinositide 3-kinase family in immune cells. *Annu. Rev. Immunol.* **31**, 675–704 (2013).
48. T. W. Hand, W. Cui, Y. W. Jung, E. Sefik, N. S. Joshi, A. Chandele, Y. Liu, S. M. Kaech, Differential effects of STAT5 and PI3K/AKT signaling on effector and memory CD8 T-cell survival. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16601–16606 (2010).
49. M. Ben Ahmed, N. Belhadj Hmdia, N. Moes, S. Buysse, M. Abdeladhim, H. Louzir, N. Cerf-Bennussan, IL-15 renders conventional lymphocytes resistant to suppressive functions of regulatory T cells through activation of the phosphatidylinositol 3-kinase pathway. *J. Immunol.* **182**, 6763–6770 (2009).
50. M. Watanabe, K. D. Moon, M. S. Vacchio, K. S. Hathcock, R. J. Hodes, Downmodulation of tumor suppressor p53 by T cell receptor signaling is critical for antigen-specific CD4⁺ T cell responses. *Immunity* **40**, 681–691 (2014).
51. W. A. Freed-Pastor, H. Mizuno, X. Zhao, A. Langerød, S. H. Moon, R. Rodriguez-Barreco, A. Barsotti, A. Chicas, W. Li, A. Polotskaia, M. J. Bissell, T. F. Osborne, B. Tian, S. W. Lowe, J. M. Silva, A. L. Børresen-Dale, A. J. Levine, J. Bargonetti, C. Prives, Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell* **148**, 244–258 (2012).
52. M. L. Coleman, C. J. Marshall, M. F. Olson, RAS and RHO GTPases in G1-phase cell-cycle regulation. *Nat. Rev. Mol. Cell Biol.* **5**, 355–366 (2004).
53. A. J. Ridley, Rho family proteins: Coordinating cell responses. *Trends Cell Biol.* **11**, 471–477 (2001).
54. I. Corre, M. Gomez, S. Vielkind, D. A. Cantrell, Analysis of thymocyte development reveals that the GTPase RhoA is a positive regulator of T cell receptor responses in vivo. *J. Exp. Med.* **194**, 903–914 (2001).
55. M. Ticchioni, C. Charvet, N. Noraz, L. Lamy, M. Steinberg, A. Bernard, M. Deckert, Signaling through ZAP-70 is required for CXCL12-mediated T-cell transendothelial migration. *Blood* **99**, 3111–3118 (2002).
56. M. Deckert, C. Moon, S. Le Bras, The immunological synapse and Rho GTPases. *Curr. Top. Microbiol. Immunol.* **291**, 61–90 (2005).
57. L. Stowers, D. Yelon, L. J. Berg, J. Chant, Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase CDC42. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5027–5031 (1995).
58. H. Yu, D. Leitenberg, B. Li, R. A. Flavell, Deficiency of small GTPase Rac2 affects T cell activation. *J. Exp. Med.* **194**, 915–926 (2001).
59. R. Behnia, S. Munro, Organelle identity and the signposts for membrane traffic. *Nature* **438**, 597–604 (2005).
60. M. Zerial, H. McBride, Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* **2**, 107–117 (2001).
61. S. G. Roy, M. W. Stevens, L. So, A. L. Edinger, Reciprocal effects of *rab7* deletion in activated and neglected T cells. *Autophagy* **9**, 1009–1023 (2013).

62. S. Ghavami, B. Yeganeh, G. L. Stelmack, H. H. Kashani, P. Sharma, R. Cunnington, S. Rattan, K. Bathe, T. Klonisch, I. M. Dixon, D. H. Freed, A. J. Halayko, Apoptosis, autophagy and ER stress in mevalonate cascade inhibition-induced cell death of human atrial fibroblasts. *Cell Death Dis.* **3**, e330 (2012).
63. D. C. Rubinsztein, P. Codogno, B. Levine, Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat. Rev. Drug Discov.* **11**, 709–730 (2012).
64. O. Gross, C. J. Thomas, G. Guarda, J. Tschoop, The inflammasome: An integrated view. *Immunol. Rev.* **243**, 136–151 (2011).
65. L. Zitvogel, O. Kepp, L. Galluzzi, G. Kroemer, Inflammasomes in carcinogenesis and anticancer immune responses. *Nat. Immunol.* **13**, 343–351 (2012).
66. G. Gruenbacher, H. Gander, O. Nussbaumer, W. Nussbaumer, A. Rahm, M. Thurnher, IL-2 costimulation enables statin-mediated activation of human NK cells, preferentially through a mechanism involving CD56⁺ dendritic cells. *Cancer Res.* **70**, 9611–9620 (2010).
67. O. Nussbaumer, G. Gruenbacher, H. Gander, M. Thurnher, DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by $\gamma\delta$ T lymphocytes. *Blood* **118**, 2743–2751 (2011).
68. E. K. Haddad, X. Wu, J. A. Hammer 3rd, P. A. Henkart, Defective granule exocytosis in Rab27a-deficient lymphocytes from *Ashen* mice. *J. Cell Biol.* **152**, 835–842 (2001).
69. M. M. Ménager, G. Ménasché, M. Romao, P. Knapnougol, C. H. Ho, M. Garfa, G. Raposo, J. Feldmann, A. Fischer, G. de Saint Basile, Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4. *Nat. Immunol.* **8**, 257–267 (2007).
70. J. C. Stinchcombe, D. C. Barral, E. H. Mules, S. Booth, A. N. Hume, L. M. Machesky, M. C. Seabra, G. M. Griffiths, Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J. Cell Biol.* **152**, 825–834 (2001).
71. J. B. Pereira-Leal, A. N. Hume, M. C. Seabra, Prenylation of Rab GTPases: Molecular mechanisms and involvement in genetic disease. *FEBS Lett.* **498**, 197–200 (2001).
72. M. Thurnher, O. Nussbaumer, G. Gruenbacher, Novel aspects of mevalonate pathway inhibitors as antitumor agents. *Clin. Cancer Res.* **18**, 3524–3531 (2012).
73. I. S. Okoye, S. M. Coomes, V. S. Pelly, S. Czieso, V. Papayannopoulos, T. Tolmachova, M. C. Seabra, M. S. Wilson, MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. *Immunity* **41**, 89–103 (2014).
74. M. Ostrowski, N. B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, C. F. Moita, K. Schauer, A. N. Hume, R. P. Freitas, B. Goud, P. Benaroch, N. Hacohen, M. Fukuda, C. Desnos, M. C. Seabra, F. Darchen, S. Amigorena, L. F. Moita, C. Thery, Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* **12**, 19–30; sup pp 11–13 (2010).
75. P. Vantourout, A. Hayday, Six-of-the-best: Unique contributions of $\gamma\delta$ T cells to immunology. *Nat. Rev. Immunol.* **13**, 88–100 (2013).
76. A. Larocca, J. A. Child, G. Cook, G. H. Jackson, N. Russell, A. Szubert, W. M. Gregory, A. Brioli, R. G. Owen, M. T. Drayson, P. Wu, A. Palumbo, M. Boccadoro, F. E. Davies, G. J. Morgan, The impact of response on bone-directed therapy in patients with multiple myeloma. *Blood* **122**, 2974–2977 (2013).
77. C. Riganti, M. Massaia, Inhibition of the mevalonate pathway to override chemoresistance and promote the immunogenic demise of cancer cells: Killing two birds with one stone. *Oncoimmunology* **2**, e25770 (2013).
78. A. C. Hayday, $\gamma\delta$ T cells and the lymphoid stress-surveillance response. *Immunity* **31**, 184–196 (2009).
79. H. J. Gober, M. Kistowska, L. Angman, P. Jenö, L. Mori, G. De Libero, Human T cell receptor $\gamma\delta$ cells recognize endogenous mevalonate metabolites in tumor cells. *J. Exp. Med.* **197**, 163–168 (2003).
80. V. Kunzmann, E. Bauer, M. Wilhelm, $\gamma\delta$ T-cell stimulation by pamidronate. *N. Engl. J. Med.* **340**, 737–738 (1999).
81. O. Nussbaumer, G. Gruenbacher, H. Gander, J. Komuczki, A. Rahm, M. Thurnher, Essential requirements of zoledronate-induced cytokine and $\gamma\delta$ T cell proliferative responses. *J. Immunol.* **191**, 1346–1355 (2013).
82. S. A. Porcelli, C. T. Morita, R. L. Modlin, T-cell recognition of non-peptide antigens. *Curr. Opin. Immunol.* **8**, 510–516 (1996).
83. G. Gruenbacher, O. Nussbaumer, H. Gander, B. Steiner, N. Leonhartsberger, M. Thurnher, Stress-related and homeostatic cytokines regulate V γ 9V δ 2 T cell surveillance of mevalonate metabolism. *Oncoimmunology* **3**, e953410 (2014).
84. J. W. Clendening, A. Pandya, P. C. Boutros, S. El Ghamrasni, F. Khosravi, G. A. Trentin, A. Martirosyan, A. Hakem, R. Hakem, I. Jurisica, L. Z. Penn, Dysregulation of the mevalonate pathway promotes transformation. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15051–15056 (2010).
85. G. Gruenbacher, M. Thurnher, Mevalonate metabolism in cancer. *Cancer Lett.* **356**, 192–196 (2014).
86. C. Harly, Y. Guillaume, S. Nedellec, C. M. Peigné, H. Mönkkönen, J. Mönkkönen, J. Li, J. Kuball, E. J. Adams, S. Netzer, J. Déchanet-Merville, A. Léger, T. Herrmann, R. Breathnach, D. Olive, M. Bonneville, E. Scotet, Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human $\gamma\delta$ T-cell subset. *Blood* **120**, 2269–2279 (2012).
87. S. Vavassori, A. Kumar, G. S. Wan, G. S. Ramanjaneyulu, M. Cavallari, S. El Daker, T. Beddoe, A. Theodossis, N. K. Williams, E. Gostick, D. A. Price, D. U. Soudamini, K. K. Voon, M. Olivo, J. Rossjohn, L. Mori, G. De Libero, Butyrophilin 3A1 binds phosphorylated antigens and stimulates human $\gamma\delta$ T cells. *Nat. Immunol.* **14**, 908–916 (2013).
88. A. Sandstrom, C. M. Peigné, A. Léger, J. E. Crooks, F. Konczak, M. C. Gesnel, R. Breathnach, M. Bonneville, E. Scotet, E. J. Adams, The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human V γ 9V δ 2 T cells. *Immunity* **40**, 490–500 (2014).

Acknowledgments: We thank W. Horninger for continuous support. **Funding:** The authors have support from Oncotyrol, a center for personalized cancer medicine. The competence center Oncotyrol is funded within the scope of the Competence Centers for Excellent Technologies (COMET) through Bundesministerium für Verkehr, Innovation und Technologie, Bundesministerium für Wissenschaft, Forschung und Wirtschaft, the province of Salzburg, and the Tiroler Zukunftsstiftung/Standortagentur Tirol. The COMET Program is conducted by the Austrian Research Promotion Agency (FFG). This work was also supported by the Austrian Cancer Society/Tirol. We appreciate the participation of the TILAK (Tiroler Landeskrankenanstalten) hospital holding company, which serves as a partner in the Oncotyrol research program.

Submitted 29 September 2014

Accepted 13 March 2015

Final Publication 31 March 2015

10.1126/scisignal.2005970

Citation: M. Thurnher, G. Gruenbacher, T lymphocyte regulation by mevalonate metabolism. *Sci. Signal.* **8**, re4 (2015).

T lymphocyte regulation by mevalonate metabolism

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Sci. Signal. **8** (370), re4.
DOI: 10.1126/scisignal.2005970

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