




Review

The role of lipid metabolism in shaping the expansion and the function of regulatory T cells

Alessandra Pinzon Grimaldos^{1,†}, Simone Bini^{2,†}, Ilenia Pacella¹, Alessandra Rossi¹,
Alessia Di Costanzo², Ilenia Minicocci², Laura D'Erasmio² , Marcello Arca² and Silvia Piconese^{1,3,4,*} 

¹Department of Internal Clinical, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy

²Department of Translational and Precision Medicine, Sapienza University of Rome, Policlinico Umberto I, Rome, Italy

³Laboratory affiliated to Istituto Pasteur Italia Fondazione Cenci Bolognetti, Rome, Italy

⁴Neuroimmunology Unit, Fondazione IRCCS Santa Lucia, Rome, Italy

†These authors contributed equally.

*Correspondence: Silvia Piconese, Department of Internal Clinical, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy.
Email: silvia.piconese@uniroma1.it

Summary

Metabolic inflammation, defined as a chronic low-grade inflammation, is implicated in numerous metabolic diseases. In recent years, the role of regulatory T cells (Tregs) as key controllers of metabolic inflammation has emerged, but our comprehension on how different metabolic pathways influence Treg functions needs a deeper understanding. Here we focus on how circulating and intracellular lipid metabolism, in particular cholesterol metabolism, regulates Treg homeostasis, expansion, and functions. Cholesterol is carried through the bloodstream by circulating lipoproteins (chylomicrons, very low-density lipoproteins, low-density lipoproteins). Tregs are equipped with a wide array of metabolic sensors able to perceive and respond to changes in the lipid environment through the activation of different intracellular pathways thus conferring to these cells a crucial metabolic and functional plasticity. Nevertheless, altered cholesterol transport, as observed in genetic dyslipidemias and atherosclerosis, impairs Treg proliferation and function through defective cellular metabolism. The intracellular pathway devoted to the cholesterol synthesis is the mevalonate pathway and several studies have shown that this pathway is essential for Treg stability and suppressive activity. High cholesterol concentrations in the extracellular environment may induce massive accumulation of cholesterol inside the cell thus impairing nutrients sensors and inhibiting the mevalonate pathway. This review summarizes the current knowledge regarding the role of circulating and cellular cholesterol metabolism in the regulation of Treg metabolism and functions. In particular, we will discuss how different pathological conditions affecting cholesterol transport may affect cellular metabolism in Tregs.

Keywords: Treg, lipoproteins, cholesterol, atherosclerosis, dyslipidemia

Abbreviations: AMPK: AMP-activated protein kinase; FOXp3: Forkhead Box P3; FH: familial hypercholesterolemia; FHBL: familial hypobetalipoproteinemia; HDL: high-density lipoprotein; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; LCFA: long chain fatty acids; LDL: low-density lipoprotein; LDLR: LDL receptor; LKB1: liver kinase B1; mTOR: mammalian target of rapamycin; SREBP: sterol regulatory element-binding protein; Tconv: conventional T cell; Treg: regulatory T cell; TCR: T-cell receptor; VAT: visceral adipose tissue; VLDL: very low-density lipoprotein.

Introduction

Tregs as key controllers of metabolic inflammation

Metabolic inflammation (metainflammation) is defined as a chronic low-grade inflammatory state, established by inflammatory cells, and induced by alterations in metabolism [1]. Metainflammation has been observed in obese individuals without infections and is associated with the development of type 2 diabetes (T2D) [2], nonalcoholic fatty liver disease (NAFLD) [2], and atherosclerosis [3].

A deep connection exists between metabolic disorders and the immune cell metabolism. Indeed, during metabolic diseases, all the pathways sensing nutrients such as glucose, amino acids, and lipids are commonly deregulated [4]. Recently, immunologists have started considering the importance of metabolism in T-cell homeostasis and functions,

thanks to a considerable number of findings in the area of immunometabolism [5].

Based on these considerations, here we are going to focus on how whole-body and cellular cholesterol metabolism influences regulatory T cells (Tregs), a specialized CD4⁺ T-cell subset necessary for the maintenance of self-tolerance, immune homeostasis, and repair functions [6–8]. Tregs are characterized by the expression of the transcription factor Forkhead Box P3 (FOXP3) that is recognized as the master gene for their development and function [9–11]. Murine models with congenital Treg deficiency develop a lethal autoimmunity phenotype [12]. Recently, Tregs have been described as the major sensor of the whole-body metabolic status due to their high sensitivity to external nutrient composition [13]. Also, increasing evidence emphasizes the role of Tregs in conditions of metabolic inflammation such as atherosclerosis, which

might be considered as a chronic inflammatory disease of the arterial walls characterized by the accumulation of cholesterol and the infiltration of immunocompetent cells [14]. It is well known that cholesterol contributes to the development of atherosclerosis because it accumulates in the intima and undergoes oxidative modification activating endothelial, smooth muscle and immune cells [15]. Both Treg number and activity are impaired in atherosclerosis thus potentially contributing to development of systemic inflammation [16–18]. To this regard, obesity, namely visceral obesity, represents a good example on how the derangement of lipid metabolism may promote metaflammation. Indeed, it has been reported that the frequency of Tregs decreases dramatically in proportion with increasing obesity in adipose tissue in both murine models and human subjects [19]. Mice fed with high-fat diet (HFD) present a drastic reduction of visceral adipose tissue (VAT) Tregs, which also associates with an inflammatory state [19]. In line with data from murine models, the Treg number in VAT and peripheral blood decreases in obese diabetic adults and children [20, 21]. In addition, obese patients with insulin resistance display reduced Treg number in their VAT as compared with lean controls, and Treg transfer can improve insulin resistance [22].

Lipoproteins and immunity

Circulating lipoproteins and the immune system

Cholesterol is a structural molecule essential for all cellular membranes, making its biosynthesis and regulatory pathways ubiquitous across cell types, including immune cells [23]. Cholesterol is carried through the bloodstream by complex structures called lipoproteins. Different lipoproteins can carry cholesterol and triglycerides either to the peripheral organs through chylomicrons, very low-density lipoproteins (VLDLs) and low-density lipoproteins (LDLs) or inversely, from peripheral organs to the liver (mainly through high-density lipoproteins, HDLs) [24].

Chylomicrons

Chylomicrons are the largest circulating lipoproteins, involved in the transport of dietary triglycerides and cholesterol from the intestine to peripheral tissues and liver. The structural component of these particles is the apolipoprotein B-48 and other functional apolipoproteins of the apoA-I, apoA-II, apoA-IV, apoA-V, apoC-II, apoC-III, and apoE series [24]. Once secreted, the half-life of chylomicrons is very short (around 5 h) [24] and they reach systemic circulation through lymphatics, thus bypassing the liver and facilitating nutrient delivery to the skeletal muscle and adipose tissue [25]. The triglycerides content of chylomicrons is hydrolyzed through the activity of lipoprotein lipase (LPL) allowing the delivery of free fatty acids to muscle and adipose tissue. LPL, an enzyme mainly expressed in the skeletal muscle and adipose tissue, is regulated by both apolipoproteins carried by the chylomicrons and circulating factors such as angiopoietin-like proteins (ANGPTL3, ANGPTL4, and ANGPTL8) [26]. As a result of chylomicron delipidation, a new particle called a chylomicron remnant is formed. Chylomicron remnants are finally cleared by the liver, where they deliver cholesterol and triglycerides [25].

Though short-lived, chylomicrons show several interactions with the immune system. De Sanctis *et al.* found that interleukin-2-activated T lymphocytes and NK cells

are both able to internalize chylomicrons and this increases their proliferative response [27]. In addition, human macrophages produce the LPL protein thus potentially contributing to chylomicron clearance [28]. Interestingly, LPL secretion shows a steep reduction when human macrophages are stimulated *in vitro* with interferon (IFN) gamma, suggesting that, during inflammation processes, chylomicron clearance is impaired thus favoring dyslipidemia and atherosclerosis [28].

Very low-density lipoproteins

VLDL particles are produced by the liver and are rich in triglycerides. They contain apoB-100, apoC-I, apoC-II, apoC-III, and apoE. Apolipoprotein B-100 is the structural core protein. It contains several highly hydrophobic areas that serve as strong lipid-binding domains and are necessary for the assembly and secretion of VLDLs. After removal of their triglyceride content by LPL activity, expressed in the skeletal muscle and adipose tissue, VLDL remnants (called IDLs) are further metabolized to LDLs [24, 29].

Like chylomicrons, VLDLs can be internalized by activated lymphocytes and NK cells with a proliferative response [24, 27]. The greatest impact in the immune system is shown by the VLDL-derived LDLs.

Low-density lipoproteins

LDL particles are the main cholesterol carriers in the blood. They are constituted by a single apoB-100 molecule folded around a hydrophobic core and surrounded by a single layer of phospholipids. Seventy percent of circulating LDLs are cleared by the liver, while the remaining part is cleared by peripheral organs through the specific LDL receptor (LDLR). Plasma concentration of LDL is highly dependent on LDLR activity: low LDLR activity corresponds to a longer LDL particle half-life and higher plasma levels [30]. Normal concentrations of plasma LDLs range from 2.6 to 3.4 mmol/l, and elevated concentrations of LDL are tightly associated with atherosclerotic cardiovascular disease. Conversely, the reduction of LDL levels are directly proportional to reduction of cardiovascular risk, which becomes minimal when LDL levels are below 1.4 mmol/l [31]. Cells of the immune system also express the LDLR and internalize cholesterol for metabolic purposes. T cells in particular are the greatest LDL consumers: being cholesterol crucial for T-cell activation and survival [32]. The internalization and catabolism of LDL-derived cholesterol in these cells was found to be three times faster than in B lymphocytes [33].

The LDL particles become immunogenic particles when oxidized (oxLDL), thus linking cholesterol transport and accumulation to metabolic inflammation. Interestingly, it has been recently described how LDL particles, differing in size and lipid composition, show a different susceptibility to oxidation and aggregation [34]. The pro-inflammatory mechanism of oxLDLs on endothelium is still not clear [35]. One hypothesis is that they may be recognized as damage-associated molecular patterns (DAMPs) by the Toll-like receptor 4 (TLR4) and may activate the nuclear factor- κ B (NF- κ B) pathway [24]. Then, the inducible nitric oxide synthase (iNOS) is hyperactivated while the endothelial nitric oxide synthase (eNOS) is downregulated, thus producing an imbalance in NO levels [36]. The subsequent increase in cellular oxidative stress activates the NLR family pyrin domain containing 3 (NLRP3) inflammasome [37].

Low-grade chronic vascular inflammation, determined by oxLDL accumulation in endothelial and subendothelial space, causes vascular dysfunction that manifests through increased endothelial permeability and increased leukocyte adhesion, chemokine secretion, and immune cell trafficking [38]. Both innate and adaptive immune cells, particularly dendritic cells (DCs), macrophages, and T cells, dominate the plaque environment throughout its evolution [39]. Newton *et al.* described how oxLDL may also influence T-cell differentiation into a pro-inflammatory Th1 phenotype, enhancing vascular inflammation and damage progression in the atheroma microenvironment [40].

Increasing evidence in mice and humans correlates the exposure to high levels of LDL with impaired Treg frequency, function, stability, and migration, and such alterations are thought to contribute to the progression of the inflammatory state in the arterial wall (Fig. 1). In two different mouse models, Treg number was shown to be reduced in the atherosclerotic plaque. Mor *et al.* have demonstrated that apolipoprotein E-deficient (*ApoE*^{-/-}) mice had a lower number of Tregs compared to wild-type littermates [41]; LDLR null (*Ldlr*^{-/-}) mice, fed an atherogenic diet, showed a drastic reduction of Tregs at 8 and 20 weeks of age, concomitant to the rise in the number of CD4 effector cells [42]. In humans, Tregs are present at lower proportion in the atherosclerotic plaques at all developmental phases [16]. In contrast to these pieces of evidence, some studies have shown that, in mice, dietary

hypercholesterolemia rather enhances Treg frequency *in vivo*, promoting their expansion in thymus and peripheral lymphoid organs, and inducing *de novo* differentiation in the liver [43, 44]. Mechanistically, cholesterol may stabilize lipids raft integrity facilitating T-cell receptor (TCR) dimerization and activation [43, 45], and it is well known that TCR homeostatic signaling is essential for Treg development in the thymus and in the periphery [46]. Such hepatically differentiated Tregs can be efficiently recruited into the aorta [44]; however, they may be exposed to local signals undermining their suppressive function and/or stability. Indeed, also a reduced Treg function appears to be linked to the atherogenic process [42]. Tregs may exert their atheroprotective properties by suppressing inflammation through the secretion of high levels of interleukin-10 (IL-10) and the transforming growth factor- β (TGF- β) [47]. IL-10 synthesis can inhibit inflammatory cytokine production by macrophages in the atherosclerotic plaque [48], whereas IL-10-deficient mice show increased effector T-cell infiltration and disease exacerbation [49]. Regarding Treg stability, Butcher *et al.* have demonstrated that the atherosclerotic plaque of *ApoE*^{-/-} deficient mice stimulates Treg plasticity, determining the development of a dysfunctional subset of IFN- γ -producing Tregs, called Th1-like Tregs, which express low levels of co-inhibitory molecules such as cytotoxic T-lymphocyte antigen 4 (CTLA 4) and the T-cell immunoreceptor with Ig and ITIM domains (TIGIT) [50].

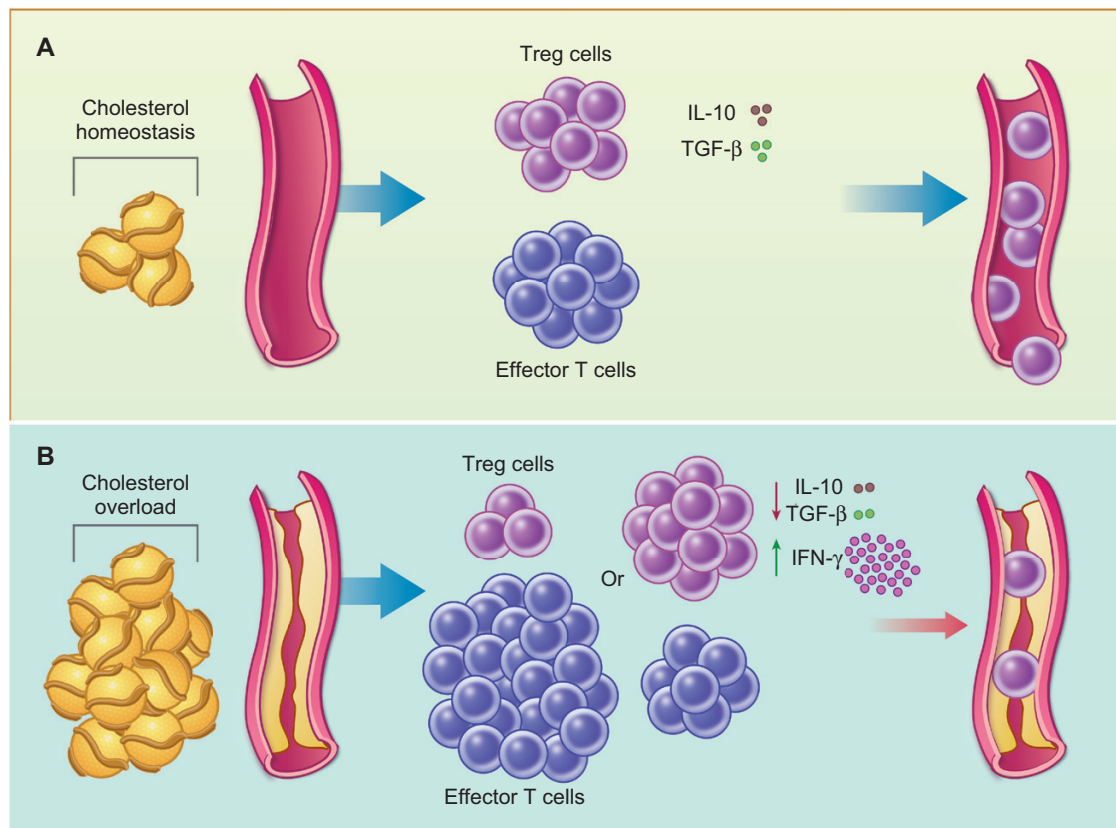


Fig. 1 Tregs are metabolic sensors and systemic cholesterol levels strongly influence Treg features. (A) In a condition of cholesterol homeostasis Tregs are proliferative, secrete cytokines, and maintain the capacity to migrate to the endothelium. (B) Pathological cholesterol overload, such as in atherosclerosis, has been shown to affect Treg frequency and function in several fashions: reduced or increased percentages, reduced ability to secrete IL-10 and TGF- β , higher differentiation into Th1-like pro-inflammatory cells, or reduced expression of selectin ligands impairing Treg capacity to migrate to the atherosclerotic plaque.

The impact of hypercholesterolemia on Treg ability to migrate into the plaque is controversial. Maganto-García *et al.* have showed a reduced migratory capacity of Tregs under prolonged hypercholesterolemia, due to a lower expression of selectin ligands [42]. Conversely, Amersfoort *et al.* have demonstrated that dyslipidemia induced a series of metabolic changes in Tregs that rather increased their capacity to migrate in the atherosclerotic plaque, suggesting that dyslipidemia-induced alterations are not due to impaired migratory capacity but to reduced suppressive function of Tregs [51].

High-density lipoproteins

HDL particles are enriched in cholesterol and phospholipids. The ability of HDL to stimulate efflux of cholesterol from peripheral tissues, to transport it into the plasma, to favor its uptake by the liver and the excretion into the bile is, comprehensively, termed reverse cholesterol transport (RCT), which is considered one of potential mechanisms by which HDLs may be anti-atherogenic. In addition, HDL particles have antioxidant, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties, which may also contribute to their ability to reduce atherosclerosis progression. Apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E are associated with these particles. ApoA-I is a major component of HDL responsible for both structure and function of this lipoprotein [24]. ApoA-I is mainly synthesized by the liver and the intestine. The main molecules involved in the efflux of cholesterol from macrophages are adenosine triphosphate (ATP)-binding cassette transporter gene A1 (*ABCA1*) and ATP-binding cassette transporter gene G1 (*ABCG1*). *ABCA1* promotes efflux of cholesterol and phospholipids onto lipid-poor apoA-1. *ABCG1* promotes cholesterol efflux from macrophage foam cells into HDL particles but the activity of *ABCG1* does not influence overall HDL levels [52]. The HDL particle also binds to the scavenger receptor class B type 1 (SR-BI), through which the cholesterol contained in the HDL particle is transported into the liver without internalization [53].

Similarly to LDLs, also HDLs show a wide range of interactions with the immune system, and most of them tend to favor an anti-inflammatory environment. Human lymphocytes are known to express the SR-BI receptor [54], which is overexpressed during clonal expansion and cell growth [55]. In a murine model, intact HDLs as well as apoA1 were found to impair antigen presentation from antigen presenting cells to T cells and thus to reduce T-cell activation, hence attenuating the pro-inflammatory reaction [56]; HDLs were also found to inhibit the interaction between activated T cells and monocytes, thus hampering the pro-inflammatory signaling carried by TNF, IL-6, IL-8, CCL3, and CCL4 [57].

Some recent evidence in mice and humans has highlighted the crucial role of HDL particles in promoting Treg survival and proliferation [58, 59]. Tregs are able to internalize HDL particles through the SR-BI/II receptor more than conventional CD4 T cells; HDL internalization increases survival through enhancement of mitochondria activity and beta-oxidation [59]; hence, the anti-inflammatory effect associated with high levels of circulating HDLs could be mediated by enhanced Treg survival.

Genetic dyslipidemias and the immune system

Genetic dyslipidemias are heritable diseases of lipid metabolism. They include at least 25 different monogenic diseases

caused by mutations in 23 candidate genes following patterns of dominant, co-dominant, or recessive inheritance [60]. Familial hypercholesterolemia (FH) is the most common genetic hypercholesterolemia showing a co-dominant pattern of inheritance. The rarest form is homozygous familial hypercholesterolemia (HoFH) (OMIM #143890) affecting 1 over 300 000 individuals in the general population [61]. Typically, it is caused by biallelic mutations in the gene coding for the LDLR. Less frequently, the HoFH phenotype is caused by biallelic mutations in other two genes that also regulate plasma clearance of LDL: the *APOB* gene and the *PCSK9* gene [62]. In addition, HoFH may also show a recessive pattern of inheritance, the so-called autosomal recessive hypercholesterolemia (ARH), which is due to mutations in the adaptor protein, *LDLRAP1* [63, 64]. The heterozygous form of familial hypercholesterolemia (HeFH) is far more common, affecting 1:250–1:300 individuals. These patients have typically increased levels of plasma cholesterol (with typical LDL cholesterol concentration > 190 mg/dl or 4.9 mmol/l). Mild hypercholesterolemic patients in the absence of a monogenic condition might suffer from polygenic hypercholesterolemia, caused by the accumulation of deleterious single-nucleotide polymorphisms (SNPs) in genes involved in cholesterol metabolism [65, 66].

Among dyslipidemias, low-cholesterol syndromes, such as the familial type 1 (FHBL1) and type 2 (FHBL2) hypobetalipoproteinemias, are far less common [67]. These conditions are characterized by a substantial reduction of both LDL and serum triglycerides. FHBL1 patients carry loss-of-function mutations in the *APOB* gene or in the microsomal triglyceride transfer protein (*MTP*) gene. Therefore, the intestine and the liver are unable to produce chylomicrons or VLDLs, respectively, resulting in lipid malabsorption, steatorrhea, and high-grade liver steatosis and fibrosis [68]. Individuals affected by FHBL2 have a loss-of-function mutation in the *ANGPTL3* gene, determinant of the hypobetalipoproteinemia phenotype and responsible for a relevant reduction in the cardiovascular risk of these individuals [69, 70].

Although cholesterol and serum lipoproteins interact widely with the immune system, little is known about the relationship between dyslipidemias and the immune system, being most of the published studies limited to FH patients. Moreover, the knowledge about the impact of rarer dyslipidemic conditions are almost completely lacking. A summary of published evidence concerning the immune system activity in genetic dyslipidemias are reported in Table 1.

The immune system in FH

As already mentioned, FH is characterized by markedly increased concentrations of LDL particles, which are able to interact with T cells and monocytes [70]. Fadini *et al.* found that hypercholesterolemic patients show an increased proportion of pro-inflammatory monocytes expressing CD68 and CCR2 markers, and their number was directly correlated with LDL-c levels [81]. A similar immune phenotype was found in children suffering from FH. In this case, the imbalance in the monocyte phenotype and monocytoysis was modulated by the HDL-c levels. Indeed, the shift toward pro-inflammatory monocytes was only present in children having HDL-c < 1.3 mmol/l (or 50 mg/dl). Composition of lymphocyte populations was comparable, except for a trend toward increased

Table 1. Immune system alterations in genetic dyslipidemias

Lipid profile alteration	Disease	Gene(s)	Effect on immune system	Ref.
↑ LDL-c	Familial hypercholesterolemia	<i>LDLR</i> <i>PCSK9</i> <i>LDLRAP1</i> <i>APOB</i>	↑ M1/M2 ratio Th1-like inflammation ^a ↑ Treg number ^a ↓ Treg suppressive function ^a	[71, 72]
	Sitosterolaemia	<i>ABCG5</i> <i>ABCG8</i>	↑ Sitosterol accumulation in macrophages, macrophage death, and vascular inflammation ^b	[73, 74]
	Lysosomal-acid lipase deficiency	<i>LIPA</i>	Impaired T-cell maturation and function ↑ regulatory T-cell ratio ↑ IL-2, IFN- γ , IL-4 ^b	[75]
↓ LDL-c	Abetalipoproteinemia	<i>APOB</i>	Impaired function of CD1-mediated antigen presentation ^b	[76]
	Familial hypobetalipoproteinemia type 1	<i>MTTP</i>		
	Familial hypobetalipoproteinemia type 2	<i>ANGPTL3</i>	Reduced population of hematopoietic stem cells in the bone marrow ^b	[76]
↑ Chylomicrons	Familial chylomicronemia syndrome	<i>LPL</i> <i>APOC2</i> <i>APOC5</i> <i>LMF1</i> <i>GPIHBP1</i>	Reduced myelopoiesis and adipose macrophage lipid content ^b	[77, 78]
↑ HDL-c	Cholesteril ester transfer protein deficiency	<i>CETP</i>	No effect on macrophage phenotype or infiltration into the plaque ^b	[79]
↓ HDL-c	Tangier disease	<i>ABCA1</i>	Increased cholesterol content in myeloid cells, activation of NLRP3 inflammasome, and increased IL-1 and IL-18 levels ^b	[80]
	Lecitin:cholesteril ester acil transferase	<i>LCAT</i>	NA	

NA, literature not available.

^aHuman studies.

^bAnimal model studies.

CD4 T lymphocytes in FH [82]. Narverud *et al.* have recently published an mRNA profiling of peripheral mononuclear cells from FH children. Interestingly, the authors found that, among the 587 genes screened, 176 were found to be significantly deregulated, in particular those involved in T- and B-cell activation and IFN signaling. The vast majority of the deregulated genes were associated with the plasma concentration of total and LDL cholesterol as well as with oxLDL. Moreover, statin therapy was found to revert the deregulation of 13 of these genes [71].

Finally, Bonacina *et al.* have recently demonstrated that FH patients show increased frequencies but decreased suppressive function of circulating Tregs [72]. Moreover, they proved that, in a murine model of hypercholesterolemia (*Ldlr*^{-/-}), the adoptive transfer of Tregs, engineered to selectively migrate to the atherosclerotic plaque, reduces atherosclerosis progression and plaque inflammation in the aorta. This study demonstrates that induction of tolerance in the plaque microenvironment reduces its progression despite the high levels of plasma LDLs, highlighting the importance of the immune function in the progression of atherosclerosis [72].

Cellular metabolism of cholesterol in T cells

Cholesterol metabolism in T cells

In recent years, an increasing number of findings have highlighted the link between intracellular metabolism and

immune cell function and dynamics. This intricate connection is known as ‘immunometabolism’ [5]. However, why T cells adopt specific metabolic programs is still unclear. Tregs can be considered a peculiar T-cell subset that is highly sensitive to the microenvironment, thanks to the wide array of immune and metabolic sensors, which allow Tregs to adapt to perturbations and external cues.

Different stages of T-cell activation can be characterized by different metabolic states. Activated T cells are highly glycolytic, and the mammalian target of rapamycin (mTOR) plays an important role in supporting the glycolytic–lipogenic metabolism [83]. The PI3K/AKT/mTOR axis is the major sensor of nutrient availability and has the critical role of promoting T-cell differentiation, homeostasis, and effector functions inducing the switch from quiescence to a metabolically active state. In conditions of poor nutrient availability, AMP-activated protein kinase (AMPK) is activated and inhibits mTOR and, thereby, a variety of biosynthetic pathways of fatty acid and cholesterol synthesis [84].

Our understanding of how cholesterol metabolism within immune cells is connected to the immune response is just beginning to emerge. Cholesterol and its derivatives are crucial to maintain plasmatic membrane fluidity, lipid raft formation, and downstream TCR signaling [45, 85]. The metabolic pathway responsible for the intracellular cholesterol availability is the mevalonate pathway, which is essential for multiple cellular processes. The third step of the mevalonate

pathway, catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), an endoplasmic reticulum resident enzyme, is generally regarded as the rate-limiting step in cholesterol synthesis [86]. This enzyme catalyzes HMG-CoA conversion to mevalonate and is inhibited by statins such as lovastatin, mevastatin, pravastatin, simvastatin, or synthetic statins [87].

The mevalonate pathway, in addition to cholesterol, generates the isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) [88]. These are the substrates of posttranslational lipid modification of cellular proteins, named prenylation, mediated by the farnesyltransferase (FT) and geranyl-geranyltransferase type 1 (GGTI). Prenylation is a lipid modification involving the covalent addition of farnesyl or, more commonly, geranyl to prenylated proteins, which plays crucial roles in cellular processes such as protein–protein interactions, intracellular trafficking, and signal transduction [89].

A tight link exists between intracellular lipid metabolism and T-cell activation, which requires a transcriptional reprogramming [90]. During T-cell activation, liver X receptors (LXRs), transcriptional factors that control different target genes involved in the efflux, transport, and excretion of cholesterol [91, 92], are downregulated, providing a proliferative advantage to T cells [90]. Concomitantly to the downregulation of LXRs, sterol regulatory element-binding proteins (SREBPs), a class of transcriptional factors involved in T-cell activation and cholesterol homeostasis [93], are upregulated [90]. SREBPs act as sensors of cholesterol content and, upon TCR stimulation, promote cholesterol biosynthesis by inducing the gene encoding for HMGCR [93]. T-cell activation is sustained not only by cholesterol, but also by other metabolites produced by the mevalonate pathway, such as FPP and GGPP. Indeed, these metabolites represent the units that are attached to the members of Ras superfamily of guanosine triphosphatases (GTPases) to mediate their membrane localization upon TCR stimulation [94]. Hence, prenylated proteins have a pivotal role in T-cell proliferation and differentiation [95].

Treg metabolism

As we have already discussed in detail in our recent review [96], Treg metabolism is highly dynamic, and its plasticity is the consequence of different origin (thymic or peripheral), type, developmental stage, and/or activity of Tregs [97]. Moreover, Tregs can display different features depending on the context in which they are studied. *In vitro*, Tregs are anergic, fail to proliferate upon TCR ligation alone, and their hyporesponsiveness can be reverted in presence of TCR ligation and high doses of interleukin-2 (IL-2) [98, 99]. Conversely, *in vivo* Tregs are highly proliferative relying on glycolysis–lipogenesis pathway [100].

Immune and metabolic signaling intersect at key molecular hubs, such as the mTOR complex [101]. Chronic overactivation or mTOR downregulation impair Treg stability and proliferation, while an oscillatory mode of mTOR activity supports Treg fitness [102, 103]. Treg expansion is also influenced by another metabolic sensor, the liver kinase B1 (LKB1). Indeed, the Treg-specific deletion of LKB1 results in reduced Treg number and function due to a defective mitochondrial metabolism [104]. Nevertheless, the downstream kinase of LKB1, AMPK, does not have a critical role for Treg

proliferation and homeostasis, and mice with AMPK-deficient Tregs show no signs of spontaneous autoimmunity [105].

Whether an improved metabolic fitness of Tregs corresponds to higher suppressive function is still matter of debate. Some data indicate that glycolysis can impair Treg suppressive function *in vivo*. In mice overexpressing the main glucose transporter GLUT1, Tregs display an increased mTOR-dependent glycolysis and high frequency but reduced suppressive activity [106]. Conversely, other data show that the mTOR-driven glycolytic–lipogenic pathway supports Treg suppressive function by promoting the expression of IFN regulatory factor 4 (IRF4) and mitochondrial metabolism [107].

At the steady state, it has been reported that Foxp3 drives a metabolic program based on fatty acid oxidation and oxidative phosphorylation (OxPhos). Indeed, Tregs have a greater capacity to uptake long chain fatty acids (LCFA) than conventional T cells (Tconvs) and their ability to oxidize fatty acids allows them avoiding lipotoxicity [108]. In this setting, Foxp3 upregulates the genes encoding for electron transport complexes and confers survival advantage to Tregs in presence of high levels of LCFA [108]. Another mechanism that Tregs exploit to tolerate high fatty acid concentration is the conversion of fatty acids into triglycerides and their storage in lipid droplets (LD). Foxp3 promotes the conversion of toxic fatty acids in triglycerides via the upregulation of the two enzymes needed for triglyceride synthesis, namely DGAT1 and DGAT2, resulting in a high content of LD, which are used to fuel mitochondrial OxPhos [109]. Fatty acids can be actively imported by Tregs from lipid-rich microenvironment. For instance, intra-tumoral Tregs enhance lipid uptake through the upregulation of the transporter CD36. The internalized lipids fuel oxidative metabolism and boost Treg stability and suppressive function by modulating mitochondrial fitness [110].

As mentioned above, Tregs are highly flexible; they adjust their metabolism in response to the environment and the stage of activity: while Treg quiescence is sustained by Foxp3 that promotes lipid-fueled OxPhos [108], Treg replication *in vivo* is supported by glycolysis–lipogenesis that is triggered by mTOR and SREBP activation and increases biomass production [100, 111, 112]. Nevertheless, Foxp3 expression is maintained during proliferation despite the mTOR-induced glycolysis activation. In addition, glycolysis is engaged when Tconvs are polarized to induced Tregs through the induction of specific FOXP3 splicing variants [113]. These findings summarize how several metabolic pathways fit together to allow Tregs adapting to different nutritional and immunological signals.

Increasing evidence highlights the role of cholesterol metabolism in Treg functions (Fig. 2). Zeng *et al.* have shown that the mevalonate pathway is the most affected pathway in a mouse model of Treg-specific mTOR deletion, and this determines altered proliferation, suppressive function, and expression of effector molecules such as CTLA-4 and ICOS, resulting in a scurfy-like autoimmune disease [112]. The key role of the mevalonate pathway in Treg homeostasis is demonstrated by the observation of a scurfy-like disease in mice bearing a Treg-restricted deletion of the HMGCR gene [114].

The upregulation of the mevalonate pathway, which is required for Treg suppressive function and stability, can be controlled by the activity of LKB1 [105, 115]. LKB1-deficient Tregs exhibit impaired suppressive activity that could be reconstituted by the addition of mevalonate. In these cells, the

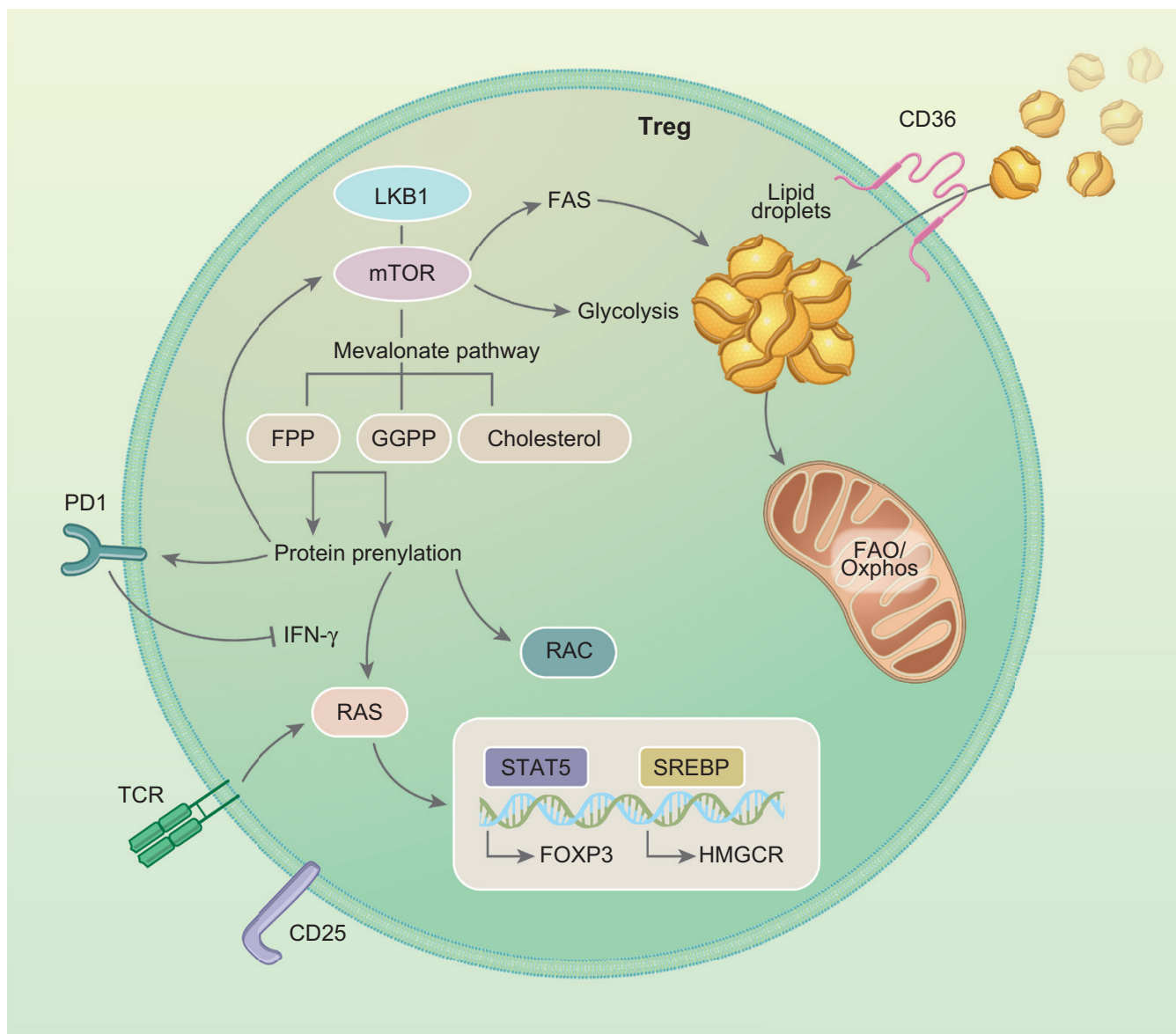


Fig. 2 Treg stability and functions heavily rely on lipid metabolism. Mevalonate pathway alteration due to the lack of LKB1-mediated signaling results in the decrease of RAC geranylation and mTOR farnesylation determining the impairment of effector Treg maintenance and differentiation, respectively. Ras geranylation sustains TCR signaling and Treg stability through STAT5 phosphorylation that induces FOXP3 expression. Moreover, in tumor, geranylation supports PD1 expression via a SREBP-dependent activation of the mevalonate pathway, preventing IFN- γ production by Tregs and sustaining their suppressive capacity. Lipids derived from fatty acid synthesis and CD36-mediated uptake can be stored as lipid droplets in the cytoplasm or can be used as substrates for fatty acid oxidation to fuel mitochondrial OxPhos. FAO, fatty acid oxidation; FAS, fatty acid synthesis; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate.

downregulation of the mevalonate pathway promotes the conversion to Th1- and Th17-like effector cells and, in support of this, purified Tregs from wild-type mice treated with statins gain the ability to produce IFN- γ [115].

Statins influence several Treg features beyond stability, as highlighted in several studies. In a standard *in vitro* suppression assay, in the presence of simvastatin, murine Tregs lost their ability to suppress, to proliferate, and to upregulate effector molecules such as CTLA4 and ICOS [112]. Furthermore, Treg proliferation and activation was impaired also *in vivo* in mice daily treated with simvastatin [112]. Opposing data are available regarding the effects of statins on human Tregs. In patients with acute coronary syndrome [116] or rheumatoid arthritis [117], statin treatment was shown to increase

Treg numbers, an event that likely contributed to symptoms amelioration. In line with these findings, atorvastatin supplementation increased Treg survival and function in a culture of human mononuclear cells *in vitro* [118, 119]. It could be proposed that, in human chronic meta-inflammatory diseases, Treg expansion may be not directly related to statin exposure, but indirectly dependent on the improvement of the inflammatory status in treated patients. However, in contrast to this idea, some data show that statins increase Treg frequency also in healthy donors [120]. Interestingly, Tregs positively correlated with HDL in statin-treated donors [120], and HDL has been shown to support Treg survival [58, 59]: these data suggest that, also in the absence of an inflammatory disease, statins may promote Treg expansion indirectly, through the

modulation of circulating lipoproteins. It may thus be difficult to discriminate whether the mevalonate pathway has a direct role in Treg expansion, and a preferential role in Tregs compared to Tconv, based on observations in statin-treated patients.

Intracellular cholesterol homeostasis must be finely balanced, and this equilibrium is regulated through the influx and efflux transport mechanisms, mediated by the scavenger receptor CD36 and the transporters ABCA1 and ABCG1 [121, 122]. LKB1 deficiency in Tregs leads to an abnormal accumulation of intracellular cholesterol due to the increased expression of CD36 and the decrease of ABC transporters, resulting in downregulation of mevalonate pathway and consequently a decrease in the levels of isoprenoids GGPP and FPP [115].

Recent data have highlighted the importance of protein prenylation in Treg fitness. Geranylgeranylation and farnesylation dictate the differentiation and the maintenance of effector Tregs through RAC geranylation and mTORC1 farnesylation, respectively [123]. These processes become particularly relevant in the context of cancer, where Tregs proliferate and acquire an effector phenotype; indeed, geranylation supports PD1 expression and represses IFN- γ production by intra-tumoral Tregs through a SREBP-dependent induction of the mevalonate pathway, while SREBP signaling is dispensable for Treg homeostasis, lipid uptake, and mitochondrial fitness under steady state [111]. It is well known that SREBP induces the mevalonate pathway [124], and indeed HMGCGR-deficient Tregs showed defective PD1 expression upon TCR stimulation, which can be restored by treatment with mevalonate or with GGPP. Thus, the axis of SREBP, mevalonate pathway, and PD1 geranylation sustains Treg stability and suppressive function and prevents Treg fragility in the tumor microenvironment [111]. The pivotal role of isoprenoids in Treg fitness has been highlighted also in a mouse model of colitis, where GGPP was shown to enhance Treg generation *in vitro* and *in vivo* through the amplification of IL-2/STAT5 signaling. Mechanistically, prenylation activates Ras, which phosphorylates ERK1/2 and this results in IL-2 production by T cells and STAT5 phosphorylation, leading to increased Treg numbers [125].

Some genetic evidence linking the mevalonate pathways and susceptibility to autoimmune and autoinflammatory diseases supports the notion that this metabolic route may play a pivotal role in Treg fitness. An SNP in the gene encoding for LKB1 has been recognized as a predisposing factor for the development of multiple sclerosis in a small human study [126]. The mevalonate kinase deficiency (MKD) encompasses a spectrum of genetic autoinflammatory disorders caused by mutations in the *MVK* gene that encodes for the mevalonate kinase. This disease can be characterized by hepatosplenomegaly, lymphadenopathy, skin rash, mucosal ulcer, abdominal pain, and severe fever attacks, as a consequence of the huge production of pro-inflammatory cytokines including IL-1 β [127]. Mechanistically, it is thought that isoprenoid reduction may increase inflammasome activation and IL-1 β release [128]. In line with this view, Politiek *et al.* have suggested that altered protein prenylation can be the pathogenic mechanism behind the MKD [129]. However, it could be also speculated that Treg-intrinsic defects in mevalonate pathway and protein prenylation may contribute to the severe inflammatory manifestations in these patients.

Several genetic defects that compromise mTOR activity have been linked to autoimmune manifestations, which are

in some cases associated to reduced Treg frequency/function [130]. The genetic deficiency of lipopolysaccharide-responsive beige-like anchor protein (LRBA), which is involved in vesicle trafficking and mTOR activation, leads to a primary immune deficiency characterized by immune dysregulation and Treg defects [131]. Also mutations affecting the CARD11–BCL10–MALT1 signalosome complex, which is required for optimal mTOR activation, are associated to reduced Treg frequencies in humans, even though this does not induce spontaneous autoimmune manifestations [132].

Connections between microenvironmental and cellular metabolism in Tregs

As highlighted above, Treg development, maintenance, and functions depend on the complex interaction with different microenvironmental and systemic cues, as a systemic metabolic alteration can negatively affect Treg features and functions [133]. A perfect example of the connection between the microenvironmental and the cellular metabolism in Tregs is represented by the metabolic syndrome (MetS), defined as the co-occurrence of certain conditions as hyperglycemia, insulin resistance, atherogenic dyslipidemia, and obesity, that contribute to the development of cardiovascular disease and T2D [134]. Obesity is the major hallmark of MetS. The first evidence connecting Treg fitness and metabolism to adipose tissue dysfunctions came from the study of Feuerer *et al.*, who examined adipose tissue-resident Tregs in lean and obese mice: Tregs preferentially accumulated in healthy VAT of lean mice, thanks to the Treg response to local antigens and to the presence of specific cytokines and adipokines promoting Treg migration and survival; conversely, Tregs frequency was dramatically reduced in VAT of obese mice [19]. The peculiar program and activity of VAT Tregs are dependent on PPAR- γ , a nuclear factor related to lipid rearrangement in adipocytes [135]. Interestingly, PPAR- γ agonism induces VAT Treg expansion in obese mice, and this event is associated to a higher accumulation of lipids in VAT Tregs [135]. In humans, the scenario is more complicated, as some studies have shown increased Treg percentages or higher FOXP3 mRNA levels in the VAT of obese compared to lean individuals [22, 136–138]. A functional assessment of these cells would help understanding whether they display qualitative defects, in their suppressive ability and/or stability, in that microenvironment.

The reduction of Treg frequency is a hallmark of obesity and T2D, and these conditions are characterized by the establishment of a chronic low-grade inflammation with high concentrations of leptin and adipocytokines. Leptin is an adipokine principally produced by adipose tissue, which controls food intake, systemic metabolism, and Treg functions [139, 140]. Human and murine Tregs produce leptin and express the leptin receptor, and leptin neutralization with a monoclonal antibody reverses human Treg anergic state *in vitro*; *in vivo*, the genetic leptin deficiency determines a higher Treg frequency compared to wild-type mice [141]. Mechanistically, obesity increases the concentration of circulating leptin leading to mTOR upregulation that, in turn, affects Treg proliferation [142]. Opposite to obesity, starvation induces a systemic leptin reduction [143], results in decreased mTOR activity, and thus promotes Treg expansion [102]. Therefore, the systemic metabolic pressure, defined as a condition of prolonged nutritional overload that determines an elevated body mass index and overproduction of adipocytokines, can be linked to the

development of autoimmune disorders in industrialized countries, possibly through a chronic limitation of Treg fitness and functions. Indeed, some studies have revealed positive associations between elevated body mass index and the development and/or progression of several autoimmune diseases [144–146].

Dietary fluctuations, which occur during a balanced diet, are associated with the oscillatory mode of mTOR activity, which is essential to ensure Treg homeostasis and proliferation [102]. During nutritional overload and obesity, chronic mTOR activation due to the constantly high level of calories and nutrients results in the impairment of Treg number and functionality, determining the accumulation of autoreactive T cells that leads to the development of autoimmunity and chronic inflammation. In support of this, an elevated body mass index is a fundamental predisposing factor for the development of type 1 diabetes and multiple sclerosis in youth. In the latter case, it has been demonstrated that, in multiple sclerosis patients, impaired Treg proliferation is associated with the activation of mTOR [147].

Conclusions

We have probably only started understanding the multiple interactions between immunity and lipid metabolism. The available evidence points to a role for nutrients in shaping the metabolism of immune cells not only by directly fueling certain metabolic routes, but also acting as signaling molecules that promote a coordinated molecular, metabolic, and functional reprogramming. The latter activity is guaranteed by the presence of molecular hubs working as metabolic rheostats, such as mTOR and SREBP [101], and of cellular platforms that are exceptionally sensitive to external variations, such as the Tregs [13]. A deeper understanding of these events may come from the study of genetic dyslipidemias characterized by extreme phenotypes and may pave the way for the development of novel immunotherapeutic approaches that integrate metabolic interventions.

Acknowledgements

Not applicable.

Funding

This work was funded by Sapienza Università di Roma - Progetti di Ricerca di Ateneo (2020 RP120172A7CBD322 to S.P. and 2019 RG11916B50C85210 to M.A.); Associazione Italiana per la Ricerca sul Cancro (IG-2017 19784 to S.P.); Ministry of Education, University and Research (MIUR) [Progetti di Ricerca di Interesse Nazionale (PRIN) Grant 2017 Prot. 2017K7FSYB to S.P.]; Italian Ministry of Health (GR-2019-12368551 to S.P.).

Conflict of interest

The authors declare no conflict of interests.

Author contributions

S.P. defined the structure and content of the manuscript and supervised the manuscript drafting. A.P.G. and S.B. wrote the first draft. A.P.G. prepared the figures. S.B. prepared the table. I.P., A.R., A.D.C., I.M., L.D., S.P., and M.A. revised the

manuscript providing relevant conceptual contribution. All authors read and approved the final paper.

Data availability

This manuscript does not contain original data.

References

- Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. *Nature* 2017, 542, 177–85.
- Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003, 112, 1821–30.
- Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol* 2013, 13, 709–21.
- Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. *Nature* 2015, 517, 302–10.
- O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* 2016, 16, 553–65.
- Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008, 133, 775–87.
- Mathur AN, Zirik B, Boothby IC, et al. Treg-cell control of a CXCL5-IL-17 inflammatory axis promotes hair-follicle-stem-cell differentiation during skin-barrier repair. *Immunity* 2019, 50, 655–67.e4.
- Li J, Tan J, Martino MM, Lui KO. Regulatory T-cells: potential regulator of tissue repair and regeneration. *Front Immunol* 2018, 9, 585.
- Gavin MA, Rasmussen JP, Fontenot JD, et al. Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* 2007, 445, 771–5.
- Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003, 299, 1057–61.
- Nomura T, Sakaguchi S. Foxp3 and Aire in thymus-generated Treg cells: a link in self-tolerance. *Nat Immunol* 2007, 8, 333–4.
- Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurf, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001, 27, 68–73.
- De Rosa V, La Cava A, Matarese G. Metabolic pressure and the breach of immunological self-tolerance. *Nat Immunol* 2017, 18, 1190–6.
- Baardman J, Lutgens E. Regulatory T cell metabolism in atherosclerosis. *Metabolites* 2020, 10, 279. doi:10.3390/metabo10070279.
- Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol* 2011, 12, 204–12.
- de Boer OJ, van der Meer JJ, Teeling P, van der Loos CM, van der Wal AC. Low numbers of FOXP3 positive regulatory T cells are present in all developmental stages of human atherosclerotic lesions. *PLoS One* 2007, 2, e779.
- Heller EA, Liu E, Tager AM, et al. Chemokine CXCL10 promotes atherogenesis by modulating the local balance of effector and regulatory T cells. *Circulation* 2006, 113, 2301–12.
- Foks AC, Lichtman AH, Kuiper J. Treating atherosclerosis with regulatory T cells. *Arterioscler Thromb Vasc Biol* 2015, 35, 280–7.
- Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* 2009, 15, 930–9.
- Zeng C, Shi X, Zhang B, et al. The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications. *J Mol Med (Berl)* 2012, 90, 175–86.
- Łuczyński W, Grubczak K, Moniuszko M, Głowińska-Olszewska B, Bossowski A. Elevated levels of Th17 cells in children with central obesity. *Scand J Clin Lab Invest* 2015, 75, 595–601.
- Eller K, Kirsch A, Wolf AM, et al. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. *Diabetes* 2011, 60, 2954–62.

23. Aguilar-Ballester M, Herrero-Cervera A, Vinue A, Martinez-Hervas S, Gonzalez-Navarro H. Impact of cholesterol metabolism in immune cell function and atherosclerosis. *Nutrients* 2020, 12.
24. Feingold KR. Introduction to lipids and lipoproteins. In: Feingold KR, Anawalt B, Boyce A, et al. (eds), *Endotext*. South Dartmouth, MA, 2000.
25. Olivecrona G. Role of lipoprotein lipase in lipid metabolism. *Curr Opin Lipidol* 2016, 27, 233–41.
26. Bini S, D'Erasmus L, Di Costanzo A, Minicocci I, Pecce V, Arca M. The interplay between angiopoietin-like proteins and adipose tissue: another piece of the relationship between adiposopathy and cardiometabolic diseases? *Int J Mol Sci* 2021, 22.
27. De Sanctis JB, Blanca I, Bianco NE. Effects of different lipoproteins on the proliferative response of interleukin-2-activated T lymphocytes and large granular lymphocytes. *Clin Sci (Lond)* 1995, 89, 511–9.
28. Jonasson L, Hansson GK, Bondjers G, Noe L, Etienne J. Interferon-gamma inhibits lipoprotein lipase in human monocyte-derived macrophages. *Biochim Biophys Acta* 1990, 1053, 43–8.
29. D'Erasmus L, Di Costanzo A, Gallo A, Bruckert E, Arca M. ApoCIII: a multifaceted protein in cardiometabolic disease. *Metabolism* 2020, 113, 154395.
30. Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009, 29, 431–8.
31. D'Erasmus L, Bini S, Arca M. Rare treatments for rare dyslipidemias: new perspectives in the treatment of homozygous familial hypercholesterolemia (HoFH) and familial chylomicronemia syndrome (FCS). *Curr Atheroscler Rep* 2021, 23, 65.
32. Panezai J, Bergdahl E, Sundqvist KG. T-cell regulation through a basic suppressive mechanism targeting low-density lipoprotein receptor-related protein 1. *Immunology* 2017, 152, 308–27.
33. Sanghvi A, Warty V. Differences in the binding, internalization and catabolism of low-density lipoprotein between normal human T and B lymphocytes. *Biochem J* 1985, 227, 397–404.
34. Ruuth M, Nguyen SD, Vihervaara T, et al. Susceptibility of low-density lipoprotein particles to aggregate depends on particle lipidome, is modifiable, and associates with future cardiovascular deaths. *Eur Heart J* 2018, 39, 2562–73.
35. Theofilis P, Sagris M, Oikonomou E, Antonopoulos AS, Siasos G, Tsioufis C, Tousoulis D. Inflammatory mechanisms contributing to endothelial dysfunction. *Biomedicines* 2021, 9.
36. Gliozzi M, Scicchitano M, Bosco F, et al. Modulation of nitric oxide synthases by oxidized LDLs: role in vascular inflammation and atherosclerosis development. *Int J Mol Sci* 2019, 20.
37. Hang L, Peng Y, Xiang R, Li X, Li Z. Ox-LDL causes endothelial cell injury through ASK1/NLRP3-mediated inflammasome activation via endoplasmic reticulum stress. *Drug Des Devel Ther* 2020, 14, 731–44.
38. Arnold N, Lechner K, Waldeyer C, Shapiro MD, Koenig W. Inflammation and cardiovascular disease: the future. *Eur Cardiol* 2021, 16, e20.
39. Roy P, Orecchioni M, Ley K. How the immune system shapes atherosclerosis: roles of innate and adaptive immunity. *Nat Rev Immunol* 2021.
40. Newton AH, Benedict SH. Low density lipoprotein promotes human naive T cell differentiation to Th1 cells. *Hum Immunol* 2014, 75, 621–8.
41. Mor A, Planer D, Luboshits G, et al. Role of naturally occurring CD4+ CD25+ regulatory T cells in experimental atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007, 27, 893–900.
42. Maganto-García E, Tarrío ML, Grabie N, Bu DX, Lichtman AH. Dynamic changes in regulatory T cells are linked to levels of diet-induced hypercholesterolemia. *Circulation* 2011, 124, 185–95.
43. Mailer RKW, Gisterå A, Polyzos KA, Ketelhuth DFJ, Hansson GK. Hypercholesterolemia enhances T cell receptor signaling and increases the regulatory T cell population. *Sci Rep* 2017, 7, 15655.
44. Mailer RKW, Gisterå A, Polyzos KA, Ketelhuth DFJ, Hansson GK. Hypercholesterolemia induces differentiation of regulatory T cells in the liver. *Circ Res* 2017, 120, 1740–53.
45. Molnár E, Swamy M, Holzer M, et al. Cholesterol and sphingomyelin drive ligand-independent T-cell antigen receptor nanoclustering. *J Biol Chem* 2012, 287, 42664–74.
46. Levine AG, Arvey A, Jin W, Rudensky AY. Continuous requirement for the TCR in regulatory T cell function. *Nat Immunol* 2014, 15, 1070–8.
47. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. *Front Immunol* 2012, 3, 51.
48. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991, 174, 1209–20.
49. Mallat Z, Besnard S, Duriez M, et al. Protective role of interleukin-10 in atherosclerosis. *Circ Res* 1999, 85, e17–24.
50. Butcher MJ, Filipowicz AR, Waseem TC, et al. Atherosclerosis-driven Treg plasticity results in formation of a dysfunctional subset of plastic IFN γ + Th1/Tregs. *Circ Res* 2016, 119, 1190–203.
51. Amersfoort J, Schaftenaar FH, Douna H, et al. Diet-induced dyslipidemia induces metabolic and migratory adaptations in regulatory T cells. *Cardiovasc Res* 2021, 117, 1309–24.
52. Zhao Y, Van Berkel TJ, Van Eck M. Relative roles of various efflux pathways in net cholesterol efflux from macrophage foam cells in atherosclerotic lesions. *Curr Opin Lipidol* 2010, 21, 441–53.
53. Tall AR. Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high density lipoproteins. *J Intern Med* 2008, 263, 256–73.
54. Feng H, Guo L, Wang D, et al. Deficiency of scavenger receptor BI leads to impaired lymphocyte homeostasis and autoimmune disorders in mice. *Arterioscler Thromb Vasc Biol* 2011, 31, 2543–51.
55. Xu Q, Jürgens G, Huber LA, Böck G, Wolf H, Wick G. Lipid utilization by human lymphocytes is correlated with high-density-lipoprotein binding site activity. *Biochem J* 1992, 285, 105–12.
56. Wang SH, Yuan SG, Peng DQ, Zhao SP. HDL and ApoA-I inhibit antigen presentation-mediated T cell activation by disrupting lipid rafts in antigen presenting cells. *Atherosclerosis* 2012, 225, 105–14.
57. Carpintero R, Gruaz L, Brandt KJ, et al. HDL interfere with the binding of T cell microparticles to human monocytes to inhibit pro-inflammatory cytokine production. *PLoS One* 2010, 5, e11869.
58. Ru D, Zhiqing H, Lin Z, et al. Oxidized high-density lipoprotein accelerates atherosclerosis progression by inducing the imbalance between treg and teff in LDLR knockout mice. *APMIS* 2015, 123, 410–21.
59. Rueda CM, Rodríguez-Perea AL, Moreno-Fernandez M, et al. High density lipoproteins selectively promote the survival of human regulatory T cells. *J Lipid Res* 2017, 58, 1514–23.
60. Hegele RA, Borén J, Ginsberg HN, et al. Rare dyslipidaemias, from phenotype to genotype to management: a European Atherosclerosis Society task force consensus statement. *Lancet Diabetes Endocrinol* 2020, 8, 50–67.
61. Santos RD, Gidding SS, Hegele RA, et al.; International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet Diabetes Endocrinol* 2016, 4, 850–61.
62. Bertolini S, Calandra S, Arca M, Averna M, Catapano AL, Tarugi P; Italian Study Group of Homozygous Familial Hypercholesterolemia. Homozygous familial hypercholesterolemia in Italy: clinical and molecular features. *Atherosclerosis* 2020, 312, 72–8.
63. D'Erasmus L, Di Costanzo A, Arca M. Autosomal recessive hypercholesterolemia: update for 2020. *Curr Opin Lipidol* 2020, 31, 56–61.
64. Sirinian MI, Belleudi F, Campagna F, et al. Adaptor protein ARH is recruited to the plasma membrane by low density lipoprotein (LDL) binding and modulates endocytosis of the LDL/LDL receptor complex in hepatocytes. *J Biol Chem* 2005, 280, 38416–23.

65. D'Erasmus L, Commodari D, Di Costanzo A, et al. Evolving trend in the management of heterozygous familial hypercholesterolemia in Italy: a retrospective, single center, observational study. *Nutr Metab Cardiovasc Dis* 2020, 30, 2027–35.
66. Di Costanzo A, Minicocci I, D'Erasmus L, et al. Refinement of pathogenicity classification of variants associated with familial hypercholesterolemia: implications for clinical diagnosis. *J Clin Lipidol* 2021, 15, 822–31. doi:10.1016/j.jacl.2021.10.001.
67. Arca M, D'Erasmus L, Minicocci I. Familial combined hypolipidemia: angiopoietin-like protein-3 deficiency. *Curr Opin Lipidol* 2020, 31, 41–8.
68. Lee J, Hegele RA. Abetalipoproteinemia and homozygous hypobetalipoproteinemia: a framework for diagnosis and management. *J Inherit Metab Dis* 2014, 37, 333–9.
69. Di Costanzo A, Di Leo E, Noto D, et al. Clinical and biochemical characteristics of individuals with low cholesterol syndromes: a comparison between familial hypobetalipoproteinemia and familial combined hypolipidemia. *J Clin Lipidol* 2017, 11, 1234–42.
70. Minicocci I, Santini S, Cantisani V, et al. Clinical characteristics and plasma lipids in subjects with familial combined hypolipidemia: a pooled analysis. *J Lipid Res* 2013, 54, 3481–90.
71. Narverud I, Christensen JJ, Bakke SS, et al. Profiling of immune-related gene expression in children with familial hypercholesterolemia. *J Intern Med* 2020, 287, 310–21.
72. Bonacina F, Martini E, Svecla M, et al. Adoptive transfer of CX3CR1 transduced-T regulatory cells improves homing to the atherosclerotic plaques and dampens atherosclerosis progression. *Cardiovasc Res* 2021, 117, 2069–82.
73. Bao L, Li Y, Deng SX, Landry D, Tabas I. Sitosterol-containing lipoproteins trigger free sterol-induced caspase-independent death in ACAT-competent macrophages. *J Biol Chem* 2006, 281, 33635–49.
74. Tabas I. A two-carbon switch to sterol-induced autophagic death. *Autophagy* 2007, 3, 38–41.
75. Qu P, Du H, Wilkes DS, Yan C. Critical roles of lysosomal acid lipase in T cell development and function. *Am J Pathol* 2009, 174, 944–56.
76. Zeissig S, Dougan SK, Barral DC, et al. Primary deficiency of microsomal triglyceride transfer protein in human abetalipoproteinemia is associated with loss of CD1 function. *J Clin Invest* 2010, 120, 2889–99.
77. Chang HR, Josefs T, Scerbo D, et al. Role of LpL (lipoprotein lipase) in macrophage polarization in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 2019, 39, 1967–85.
78. Chang CL, Garcia-Arcos I, Nyrén R, et al. Lipoprotein lipase deficiency impairs bone marrow myelopoiesis and reduces circulating monocyte levels. *Arterioscler Thromb Vasc Biol* 2018, 38, 509–19.
79. Josefs T, Basu D, Vaisar T, et al. Atherosclerosis regression and cholesterol efflux in hypertriglyceridemic mice. *Circ Res* 2021, 128, 690–705.
80. Westerterp M, Fotakis P, Ouimet M, et al. Cholesterol efflux pathways suppress inflammasome activation, NETosis, and atherogenesis. *Circulation* 2018, 138, 898–912.
81. Fadini GP, Simoni F, Cappellari R, et al. Pro-inflammatory monocyte-macrophage polarization imbalance in human hypercholesterolemia and atherosclerosis. *Atherosclerosis* 2014, 237, 805–8.
82. Christensen JJ, Osnes LT, Halvorsen B, et al. Altered leukocyte distribution under hypercholesterolemia: a cross-sectional study in children with familial hypercholesterolemia. *Atherosclerosis* 2017, 256, 67–74.
83. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science* 2013, 342, 1242454.
84. Huang H, Long L, Zhou P, Chapman NM, Chi H. mTOR signaling at the crossroads of environmental signals and T-cell fate decisions. *Immunol Rev* 2020, 295, 15–38.
85. Wang F, Beck-García K, Zorzini C, Schamel WW, Davis MM. Inhibition of T cell receptor signaling by cholesterol sulfate, a naturally occurring derivative of membrane cholesterol. *Nat Immunol* 2016, 17, 844–50.
86. Bloch K. The biological synthesis of cholesterol. *Science* 1965, 150, 19–28.
87. Greenwood J, Steinman L, Zamvil SS. Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. *Nat Rev Immunol* 2006, 6, 358–70.
88. McTaggart SJ. Isoprenylated proteins. *Cell Mol Life Sci* 2006, 63, 255–67.
89. Casey PJ, Seabra MC. Protein prenyltransferases. *J Biol Chem* 1996, 271, 5289–92.
90. Bensinger SJ, Bradley MN, Joseph SB, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell* 2008, 134, 97–111.
91. Tontonoz P, Mangelsdorf DJ. Liver X receptor signaling pathways in cardiovascular disease. *Mol Endocrinol* 2003, 17, 985–93.
92. Kidani Y, Bensinger SJ. Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. *Immunol Rev* 2012, 249, 72–83.
93. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997, 89, 331–40.
94. Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. *J Cell Sci* 2005, 118, 843–6.
95. Dunn SE, Youssef S, Goldstein MJ, et al. 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.
96. Pacella I, Piconese S. Immunometabolic checkpoints of Treg dynamics: adaptation to microenvironmental opportunities and challenges. *Front Immunol* 2019, 10, 1889.
97. Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. *Nat Rev Immunol* 2003, 3, 253–7.
98. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998, 188, 287–96.
99. Ng WF, Duggan PJ, Ponchel F, et al. Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. *Blood* 2001, 98, 2736–44.
100. Procaccini C, Carbone F, Di Silvestre D, et al. The proteomic landscape of human ex vivo regulatory and conventional T cells reveals specific metabolic requirements. *Immunity* 2016, 44, 406–21.
101. Valvezan AJ, Manning BD. Molecular logic of mTORC1 signalling as a metabolic rheostat. *Nat Metab* 2019, 1, 321–33.
102. Procaccini C, De Rosa V, Galgani M, et al. An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. *Immunity* 2010, 33, 929–41.
103. Sabbatini M, Ruggiero G, Palatucci AT, et al. Oscillatory mTOR inhibition and Treg increase in kidney transplantation. *Clin Exp Immunol* 2015, 182, 230–40.
104. He N, Fan W, Henriquez B, et al. Metabolic control of regulatory T cell (Treg) survival and function by Lkb1. *Proc Natl Acad Sci USA* 2017, 114, 12542–7.
105. Yang K, Blanco DB, Neale G, et al. Homeostatic control of metabolic and functional fitness of Treg cells by LKB1 signalling. *Nature* 2017, 548, 602–6.
106. Gerriets VA, Kishont RJ, Johnson MO, et al. Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat Immunol* 2016, 17, 1459–66.
107. Chapman NM, Zeng H, Nguyen TM, et al. mTOR coordinates transcriptional programs and mitochondrial metabolism of activated Treg subsets to protect tissue homeostasis. *Nat Commun* 2018, 9, 2095.
108. Howie D, Cobbold SP, Adams E, et al. Foxp3 drives oxidative phosphorylation and protection from lipotoxicity. *JCI Insight* 2017, 2, e89160.
109. Howie D, Ten Bokum A, Cobbold SP, Yu Z, Kessler BM, Waldmann H. A novel role for triglyceride metabolism in Foxp3 expression. *Front Immunol* 2019, 10, 1860.

110. Wang H, Franco F, Tsui YC, et al. CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nat Immunol* 2020, 21, 298–308.
111. Lim SA, Wei J, Nguyen TM, et al. Lipid signalling enforces functional specialization of Treg cells in tumours. *Nature* 2021, 591, 306–11.
112. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* 2013, 499, 485–90.
113. De Rosa V, Galgani M, Porcellini A, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. *Nat Immunol* 2015, 16, 1174–84.
114. Lacher SM, Bruttger J, Kalt B, et al. HMG-CoA reductase promotes protein prenylation and therefore is indispensable for T-cell survival. *Cell Death Dis* 2017, 8, e2824.
115. Timilshina M, You Z, Lacher SM, et al. Activation of mevalonate pathway via LKB1 is essential for stability of Treg cells. *Cell Rep* 2019, 27, 2948–2961.e7.
116. Ma X, Liu S, Li T, Yuan H. Intensive statin treatment ameliorate the Th17/Treg functional imbalance in patients with non-ST elevation acute coronary syndrome underwent percutaneous coronary intervention. *Clin Cardiol* 2020, 43, 379–85.
117. Tang TT, Song Y, Ding YJ, et al. Atorvastatin upregulates regulatory T cells and reduces disease activity in patients with rheumatoid arthritis. *J Lipid Res* 2011, 52, 1023–32.
118. Mausner-Fainberg K, Luboshits G, Mor A, et al. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells. *Atherosclerosis* 2008, 197, 829–39.
119. Meng X, Zhang K, Li J, et al. Statins induce the accumulation of regulatory T cells in atherosclerotic plaque. *Mol Med* 2012, 18, 598–605.
120. Rodríguez-Perea AL, Montoya CJ, Olek S, Chougnet CA, Velilla PA. Statins increase the frequency of circulating CD4+ FOXP3+ regulatory T cells in healthy individuals. *J Immunol Res* 2015, 2015, 762506.
121. Spann NJ, Glass CK. Sterols and oxysterols in immune cell function. *Nat Immunol* 2013, 14, 893–900.
122. Kidani Y, Elsaesser H, Hock MB, 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.
123. Su W, Chapman NM, Wei J, et al. Protein prenylation drives discrete signaling programs for the differentiation and maintenance of effector Treg cells. *Cell Metab* 2020, 32, 996–1011.e7.
124. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002, 109, 1125–31.
125. Pandit M, Acharya S, Gu Y, et al. Geranylgeranyl pyrophosphate amplifies Treg differentiation via increased IL-2 expression to ameliorate DSS-induced colitis. *Eur J Immunol* 2021, 51, 1461–72.
126. Boullerne AI, Skias D, Hartman EM, Testai FD, Kalinin S, Polak PE, Feinstein DL. A single-nucleotide polymorphism in serine-threonine kinase 11, the gene encoding liver kinase B1, is a risk factor for multiple sclerosis. *ASN Neuro* 2015, 7, 1–13.
127. van der Burgh R, Ter Haar NM, Boes ML, Frenkel J. Mevalonate kinase deficiency, a metabolic autoinflammatory disease. *Clin Immunol* 2013, 147, 197–206.
128. Mandey SH, Kuijk LM, Frenkel J, Waterham HR. A role for geranylgeranylation in interleukin-1beta secretion. *Arthritis Rheum* 2006, 54, 3690–5.
129. Politeiek FA, Waterham HR. Compromised protein prenylation as pathogenic mechanism in mevalonate kinase deficiency. *Front Immunol* 2021, 12, 724991.
130. Jung S, Gámez-Díaz L, Proietti M, Grimbacher B. “Immune TORopathies,” a novel disease entity in clinical immunology. *Front Immunol* 2018, 9, 966.
131. Charbonnier LM, Janssen E, Chou J, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. *J Allergy Clin Immunol* 2015, 135, 217–27.
132. Turvey SE, Durandy A, Fischer A, et al. The CARD11-BCL10-MALT1 (CBM) signalosome complex: stepping into the limelight of human primary immunodeficiency. *J Allergy Clin Immunol* 2014, 134, 276–84.
133. Hoeppli RE, Wu D, Cook L, Levings MK. The environment of regulatory T cell biology: cytokines, metabolites, and the microbiome. *Front Immunol* 2015, 6, 61.
134. Huang PL. A comprehensive definition for metabolic syndrome. *Dis Model Mech* 2009, 2, 231–7.
135. Cipolletta D, Feuerer M, Li A, et al. PPAR- γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* 2012, 486, 549–53.
136. Donninelli G, Del Cornò M, Pierdominici M, et al. Distinct blood and visceral adipose tissue regulatory T cell and innate lymphocyte profiles characterize obesity and colorectal cancer. *Front Immunol* 2017, 8, 643.
137. Pereira S, Teixeira L, Aguilar E, et al. Modulation of adipose tissue inflammation by FOXP3+ Treg cells, IL-10, and TGF- β in metabolically healthy class III obese individuals. *Nutrition* 2014, 30, 784–90.
138. Zeyda M, Huber J, Prager G, Stulnig TM. Inflammation correlates with markers of T-cell subsets including regulatory T cells in adipose tissue from obese patients. *Obesity (Silver Spring)* 2011, 19, 743–8.
139. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998, 395, 763–70.
140. Matarese G, Carrieri PB, La Cava A, et al. Leptin increase in multiple sclerosis associates with reduced number of CD4(+)CD25+ regulatory T cells. *Proc Natl Acad Sci USA* 2005, 102, 5150–5.
141. De Rosa V, Procaccini C, Cali G, et al. A key role of leptin in the control of regulatory T cell proliferation. *Immunity* 2007, 26, 241–55.
142. Marrodan M, Farez MF, Balbuena Aguirre ME, Correale J. Obesity and the risk of multiple sclerosis. the role of leptin. *Ann Clin Transl Neurol* 2021, 8, 406–24.
143. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998, 394, 897–901.
144. Ferrara-Cook C, Geyer SM, Evans-Molina C, et al.; Type 1 Diabetes TrialNet Study Group. Excess BMI accelerates islet autoimmunity in older children and adolescents. *Diabetes Care* 2020, 43, 580–7.
145. Guo X, He Z, Shao S, et al. Interaction effect of obesity and thyroid autoimmunity on the prevalence of hyperthyrotropinaemia. *Endocrine* 2020, 68, 573–83.
146. Harpsøe MC, Basit S, Andersson M, et al. Body mass index and risk of autoimmune diseases: a study within the Danish National Birth Cohort. *Int J Epidemiol* 2014, 43, 843–55.
147. Carbone F, De Rosa V, Carrieri PB, et al. Regulatory T cell proliferative potential is impaired in human autoimmune disease. *Nat Med* 2014, 20, 69–74.