Cholesterol metabolism in T cells in health and disease

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Abstract

Cholesterol is present in almost all eukaryotic cells and has many functions in health and disease. One of the cell types influenced by cholesterol are T cells. The aim of this review is to discuss the physiological and pathophysiological effects of cholesterol metabolism on T cell function and to provide an overview of the existing knowledge gaps.

Cholesterol plays an important role in T cells by contributing to T cell activation and proliferation and determining T cell differentiation. Indeed, while differentiation of T helper (Th)1 cells, Th17 cells, γδT cells, cytotoxic T lymphocytes, and CD4+ memory T cells is stimulated by an active intracellular cholesterol biosynthesis, Th2 cells and CD8+ memory T cells require a suppressed cholesterol biosynthesis pathway. Differentiation of regulatory T (Treg) cells from naive T cells requires low cholesterol biosynthesis, while reactivation of Tregs is stimulated by an active flux of the biosynthesis pathway. Diseases in which cholesterol metabolism is disrupted display an altered T cell profile. This contributes to clinical symptoms in for example hypercholesterolemia, Tangier disease, and Smith-Lemli-Opitz syndrome patients.

One of the biggest knowledge gaps on cholesterol metabolism in T cells is the relative contribution of cholesterol uptake, biosynthesis, efflux, and esterification on T cell function in health and disease. It also remains to be understood whether cholesterol biosynthesis and lipoprotein uptake are redundant mechanisms and in which way cholesterol esterification and uptake influence T cell function. Other outstanding questions include the mechanisms behind a reliance on cholesterol biosynthesis, the effects of statins on T cells, and the translation of results obtained in mice to humans.

Unravelling the full picture of cholesterol metabolism in T cells will aid in the understanding of diseases with a disrupted T cell homeostasis, as well as to the development of efficient therapies.

Keywords: cholesterol; T cells; hypercholesterolemia; Tangier; Smith-Lemli-Opitz

Layman's summary

Cholesterol is a lipid molecule, found in almost all cells of the human body. Cholesterol is important for cellular functioning in many ways, such as being a membrane constituent and a signalling molecule. One of the cell types influenced by cholesterol are T cells. T cells are the major components of the adaptive immune system. When the immune system encounters an invader, T cells become activated and start to proliferate. During proliferation, T cells differentiate into several subtypes with each their own function to combat the invader. Cholesterol plays an important role in T cell activation and proliferation. Furthermore, the amount of cholesterol synthesis in a T cell determines into which subtype the T cell differentiates.

In certain diseases cholesterol metabolism is disrupted. A well-known disease is hypercholesterolemia, also called high blood cholesterol. Hypercholesterolemia can be caused by a high-cholesterol diet or by genetic factors. In both cases, cholesterol levels in the blood stream are increased, which may lead to increased cholesterol levels inside T cells as well. In hypercholesterolemia, an increased T cell activation and proliferation is observed and the differentiation of T cells into other subtypes is altered compared to healthy humans. Among others, this can lead to unwanted inflammation, which has detrimental effects on the body. There are also rarer diseases with a disrupted cholesterol metabolism, such as Tangier disease in which cholesterol cannot be exported out of the cells, thus accumulating inside. Another rare disease is Smith-Lemli-Opitz syndrome, in which cholesterol cannot be synthesized. Both diseases alter T cell functioning, which leads to clinical symptoms.

Because T cells have important functions in the body, more research to the influences of cholesterol on T cells is necessary. It is, for example, unknown how the four pathways involved in cholesterol homeostasis (cholesterol uptake, biosynthesis, efflux, and esterification) relatively contribute to the intracellular cholesterol levels in T cells. The exact mechanisms behind a reliance on cholesterol biosynthesis also remain to be understood, as well as how statins affect T cells and if results obtained in mice can be translated to humans.

A more complete understanding of cholesterol metabolism in T cells will aid in the development of efficient therapies for diseases with a disrupted cholesterol metabolism.

1. Introduction

Cholesterol is a lipid molecule that is found in almost all eukaryotic cells. Over the last decades, the physiological and pathological pathways involving cholesterol have been increasingly studied(Schade et al., 2020). This have led to the insight that cholesterol is essential to maintain membrane fluidity, permeability, and signalling. Cholesterol is also an important building block for phospholipids and other membrane constituents, and therefore indispensable in proliferating cells(Aguilar-Ballester et al., 2020).

Defective cholesterol metabolism could, depending on the specific cause, lead to atherosclerosis, neurological impairments, and developmental delay(Luo et al., 2020). In cholesterol-related diseases, immune cells are involved. Although much attention has been paid to macrophages as being the link between cholesterol and disease, more and more research indicates that T cells play an important role too. T cells are the major components of the human immune system and play a central role in the adaptive immune response. When T cells encounter their cognate antigen, they become activated, which leads to proliferation and differentiation in order to efficiently eliminate pathogens. Cholesterol influences all these different stages of T cells(Aguilar-Ballester et al., 2020). Hence, we hypothesize that aberrant cholesterol metabolism could alter the functionality of T cells, thereby leading to disease.

This review covers the influence of physiological and pathophysiological cholesterol metabolism on T cell function. First, a short overview on cellular cholesterol homeostasis will be given. Then, findings regarding the functions of cholesterol in different stages of T cell activation and differentiation will be discussed. These functions will be linked to diseases in which cholesterol metabolism is disrupted. Finally, a future perspective will provide an overview of the outstanding questions in the field that need to be answered.

2. Cholesterol homeostasis

Cholesterol homeostasis is tightly regulated, which is important for proper cellular functioning. Cellular cholesterol homeostasis can be divided in four major processes: cholesterol biosynthesis, uptake, esterification, and efflux (Figure 1). The amount of intracellular cholesterol primarily regulates the flux of these four processes. A decrease of intracellular cholesterol levels stimulates intracellular cholesterol biosynthesis and uptake from the bloodstream to restore cholesterol levels (Figure 1, left). On the other hand, increased intracellular cholesterol levels stimulate cholesterol efflux and esterification (Figure 1, right)(Brown et al., 2018).

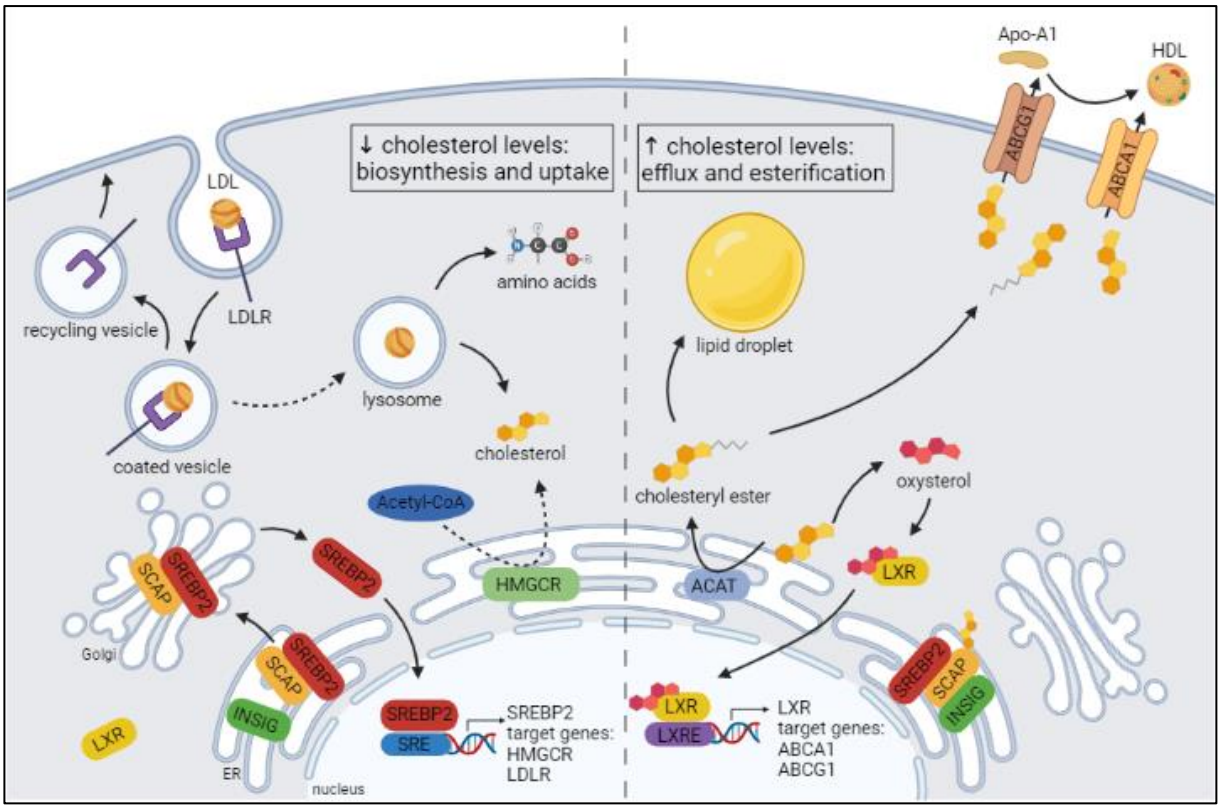

Figure 1. Schematic overview of the regulation of intracellular cholesterol homeostasis

Low intracellular cholesterol levels induce cholesterol biosynthesis and uptake (left): absence of cholesterol liberates SCAP/SREBP2 in the ER from INSIG. In the Golgi network, SREBP2 is liberated from SCAP and translocates to the nucleus. SREBP2 binds to SREs and transcribes target genes such as HMGCR and LDLR. HMGCR is the rate-limiting enzyme in cholesterol biosynthesis and converts acetyl-CoA to cholesterol in the ER. LDLR facilitates receptor-mediated endocytosis of cholesterol-containing LDL particles. Inside the cell, LDL detaches form the LDLR, which is recycled back to the plasma membrane. Within lysosomes, LDL particles release cholesterol and amino acids.

High intracellular cholesterol levels induce cholesterol efflux and esterification (right): cholesterol-derived oxysterols activate LXR, which binds to LXREs and transcribes target genes such as ABCA1 and ABCG1. ABCA1 and ABCG1 transport cholesterol and cholesteryl esters out of the cell. Apo-A1 proteins take up cholesterol which yields HDL particles. Cholesterol esterification is facilitated by ACAT within the ER and generates cholesteryl esters, which are either stored in lipid droplets or released.

Dashed lines: process involves more steps than depicted.

ABCA1/G1: ATP-binding cassette A1/G1; ACAT: acyl-CoA cholesterol acyl transferase; Apo-A1: apolipoprotein A1; ER: endoplasmic reticulum; HDL: high-density lipoprotein; HMGCR: 3-hydroxy-3-methylglutaryl CoA reductase; LDL: low-density lipoprotein; LDLR: low-density lipoprotein receptor; LXR: liver X receptor; LXRE: liver X receptor response element; SRE: sterol regulatory element; SREBP2: sterol regulatory element-binding protein 2.

Figure made with BioRender.

The transcription factor sterol regulatory element-binding protein 2 (SREBP2) plays a major role in transcribing genes involved in intracellular cholesterol biosynthesis and uptake. SREBP2 is localized in the endoplasmic reticulum (ER) together with the cholesterol sensor SREBP cleavage-activating

protein (SCAP). When intracellular cholesterol levels are high, SCAP is bound by cholesterol. Cholesterol-bound SCAP interacts with the protein insulin induced gene 1 (INSIG), which keeps the SCAP/SREBP2 complex within the ER. Upon cholesterol depletion, the inhibition of SCAP/SREBP2 by INSIG is relieved. Subsequently, SCAP/SREBP2 translocates to the Golgi network where SREBP2 is liberated. The free SREBP2 enters the nucleus where it binds as homodimer to sterol regulatory elements (SREs) of target genes(Brown et al., 2018).

Important SREBP2 target genes include those coding for 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) and the low-density lipoprotein receptor (LDLR)(Inoue et al., 1998; Luo et al., 2020). HMGCR is the rate-limiting enzyme of the cholesterol biosynthesis pathway. Together with several other enzymes, HMGCR converts acetyl-CoA to cholesterol within the ER. LDLR is the receptor responsible for uptake of cholesterol-rich low-density lipoprotein (LDL) particles. These particles are the major source of extracellular cholesterol for peripheral cells(Luo et al., 2020). Binding of LDL to the LDLR stimulates receptor-mediated endocytosis of these complexes. Inside the cell, LDL detaches from the LDLR. While the LDLR is recycled back to the plasma membrane, LDL particles are broken down inside lysosomes. The degradation of LDL in lysosomes yields amino acids and cholesterol. Cholesterol is subsequently transported to other compartments in the cell, mainly the ER(Brown et al., 2018; Hussain, 2014).

The cholesterol component of LDL particles is derived from the diet. In the gut, enterocytes absorb cholesterol and subsequently release it to the bloodstream in the form of chylomicrons. Chylomicrons are processed by the liver which releases cholesterol in very low-density lipoprotein (VLDL) particles. In the bloodstream, VLDL particles are processed to LDL particles which can be taken up by peripheral cells(Hussain, 2014).

Increased intracellular cholesterol levels stimulate cholesterol efflux and esterification to restore cholesterol levels. Cellular cholesterol efflux is regulated by the transcription factor liver X receptor (LXR). Under conditions of excess cholesterol, cholesterol is converted to several oxysterols, which are ligands of LXR. Oxysterol-bound LXR translocates to the nucleus where it binds to the liver X receptor response element (LXRE) of target genes.

Important LXR target genes are those coding for the transporters ATP-binding cassette A1 and G1 (ABCA1/G1). ABCA1 and ABCG1 transport cholesterol out of the cell where it binds to apolipoprotein A1 (Apo-A1) to yield high-density lipoprotein (HDL) particles. HDL particles are processed by the liver and intestine to synthesize bile acids or by steroidogenic organs to synthesize hormones(Armstrong et al., 2010; Bovenga et al., 2015).

Another way to lower intracellular cholesterol levels is by esterification. Cholesterol esterification consists of the addition of an alkyl group to cholesterol, which is facilitated by the enzyme acyl-CoA cholesterol acyl transferase (ACAT) in the ER. The obtained cholesteryl esters could be stored in lipid droplets inside cells or be released as constituents of lipoproteins(Bovenga et al., 2015)

All four processes involved in cholesterol homeostasis (i.e., cholesterol biosynthesis, uptake, export, esterification) are tightly regulated by feedback and feedforward mechanisms to maintain a constant level of cholesterol within cells. In this way, cellular processes in which cholesterol is needed are enabled, while toxicity by excessive levels of cholesterol is prevented(Luo et al., 2020).

Although the details of cholesterol homeostasis have been researched mainly in hepatocytes and macrophages, the mechanisms of cholesterol biosynthesis, uptake, esterification, and efflux apply to T cells as well(Aguilar-Ballester et al., 2020; Geltink et al., 2018). However, research on the relative contribution of the four different mechanisms in T cells is lacking. In other words, the relevance and efficiency of the feedback mechanisms in T cells are unclear. It also remains unidentified if only the

LDLR is responsible for LDL uptake in T cells, or if other receptors are involved as well. Furthermore, while it is known that T cells express the LDLR, the absolute mRNA and protein quantities remain undefined (Perucha et al., 2019). Recently, a large amount of RNA sequencing data in T cells has become available(Szabo et al., 2019). It would be interesting to analyse these data and determine the relative contribution of mRNA coding for the different proteins involved in T cell homeostasis, such as LDLR and HMGCR.

3. Functions of cholesterol in naive T cells

Lymphoid progenitor cells destined to become T lymphocytes migrate from the bone marrow to the thymus. For this reason, the cells are called thymus-dependent (T) cells. Inside the thymus, lymphoid progenitor cells proliferate and undergo positive and negative selection processes. Selection eventually yields different types of T cells (CD4+, CD8+), depending on the specific receptor expression. These T cells circulate throughout the body in search for their cognate antigen. Since these T cells have not yet encountered their cognate antigens with their specific T cell receptor (TCR), they are considered immature, naive T (Tn) cells(Janeway et al., 2001).

Tn cells are in a quiescent stage, which is the G0 stage of the cell cycle. In this stage, T cells rely on fatty acid oxidation and a low rate of glycolysis to fuel the citric acid cycle and perform oxidative phosphorylation for ATP generation. In the absence of cognate antigens presented by major histocompatibility complexes (MHC), it is important that Tn cells remain quiescent. Uncontrolled T cell activation, namely, could lead to autoimmunity.

To maintain quiescence in Tn cells, it is important that metabolic pathways are not fully engaged. These pathways generate reactive oxygen species (ROS), involved in the stimulation of mechanistic target of rapamycin (mTOR). Activated mTOR triggers cell cycle entry, thus activating Tn cells. Simultaneously, a small flow of metabolic processes is needed to supply Tn cells with sufficient energy to circulate through the body and prevent apoptosis. Low interactions with self-peptides, and the signalling molecules mTOR and extracellular adenosine are important in keeping this metabolic balance(Geltink et al., 2018).

Cholesterol is one of the critical metabolic pathways in Tn cells. Cholesterol contributes to the regulation of Tn cell activity, both by actions at the plasma membrane and intracellularly, which will be described in the current section.

3.1 Stabilization of T cell receptors

About 35% of the mammalian cell membrane consists of cholesterol(Pinkwart et al., 2019). One function of cholesterol at the plasma membrane is the stabilization of resting TCRs. Unstimulated TCRs of Tn cells spontaneously switch between a resting and active conformation. Constantly, 98% of the TCRs are in the resting state. This high percentage of TCRs in the resting conformation prevents unwanted antigen-independent activation. Resting TCRs are stabilized by cholesterol, which binds to them.

The interaction between cholesterol and resting TCRs is dynamic, as shown by a model developed by Swamy and collegues. At a certain moment, about 50% of resting TCRs is cholesterol-bound. The unbound resting TCRs can still switch to the active conformation, which allows for binding by antigens. Antigen binding to TCRs in the active conformation fully activates the TCR by phosphorylation. The model of Swamy et al. illustrates how cholesterol contributes to the activation threshold for TCR ligands(Swamy et al., 2016). In this way, cholesterol aids in maintaining Tn cells quiescent.

3.2 Formation of T cell receptor nanoclusters

Besides stabilizing inactive TCRs, cholesterol also contributes to TCR nanocluster formation in Tn cells. TCR nanoclusters consist of 2-30 TCRs located together with TCR monomers within the cholesterol- and sphingomyelin-rich lipid rafts(Molnár et al., 2012). Compared to monomeric TCRs, nanoclusters display an increased signalling upon binding of peptide-MHC complexes. The increased signalling is likely caused by cooperativity between TCRs. Furthermore, neighbouring TCRs in a nanocluster could prolong the binding between a TCR and peptide-MHC complex, which reinforces TCR activation(Pageon et al., 2016). Molnár and collegues showed that cholesterol binds to the β

chain of TCRs, both *in vitro* and *in vivo*. In artificial liposomes containing only two TCRs, the cholesterol-TCR binding induced TCR dimerization. *In vivo*, TCR dimers presumably cluster together to form nanoclusters(Molnár et al., 2012).

By contributing to TCR clustering, cholesterol determines the avidity (i.e., the strength of multivalent interactions) of TCR-antigen interactions. This is illustrated by cholesterol depletion, which decreases the avidity and therefore increases the threshold of TCR activation(Molnár et al., 2012). Therefore, by its role in TCR nanocluster formation, cholesterol contributes to the exit from quiescence in Tn cells upon interaction with their cognate antigens.

Several examples illustrate the role of cholesterol in setting the TCR activation threshold. Four of them will be described here. Firstly, it is known that the sensitivity for activation of T lymphocytes decreases during maturation, which is caused by disruption of TCR nanoclusters by cholesterol sulphate. Cholesterol sulphate is synthesized from cholesterol by the enzyme sulfotransferase family 2B member 1 (SULT2B1). During T cell maturation, SULT2B1 becomes upregulated, which increases the formation of cholesterol sulphate(F. Wang et al., 2016). Furthermore, since SULT2B1 also reduces LXR signalling, the intracellular cholesterol content increases(Bensinger et al., 2008), which may lead to the formation of even more cholesterol sulphate. Through binding competition, cholesterol sulphate replaces cholesterol bound to TCRβ in nanoclusters, which disorganizes TCR nanoclusters, thus repressing TCR signalling upon anti-CD3 stimulation(F. Wang et al., 2016).

Secondly, the function of cholesterol in nanocluster formation is demonstrated by the fact that CD4+ thymocytes are less sensitive for CD3 stimulation compared to CD4+ splenocytes. One of the underlying reasons for this lower sensitivity is a decreased cholesterol content within lipid rafts on the plasma membrane. Namely, CD4+ thymocytes display less HMGCR activity compared to CD4+ splenocytes, which results in less cholesterol biosynthesis. The reduction in cholesterol biosynthesis leads to a decreased lipid raft formation, thereby lowering the amount of TCR nanoclusters. Subsequently, CD3 stimulation results in less proliferation and cytokine secretion of CD4+ thymocytes compared to CD4+ splenocytes(Brumeanu et al., 2007).

Thirdly, Cheng et al. showed that the role of cholesterol in TCR nanoclusters (partially) accounts for the quicker activation of γδ T cells upon antigen recognition compared to conventional αβ T cells. γδ T cells are a distinct type of T lymphocytes and could be recognized by the presence of the γ and δ TCR chain. γδ T cells are important in the first line of defence against pathogens and they play a role in activation of adaptive immunity. The quick activation of γδ T cells is, at least partially, caused by an increased cholesterol content compared to αβ T cells. This results in an increased lipid raft and TCR nanocluster formation, which enhances TCR signalling(Cheng et al., 2013).

Fourthly, the function of cholesterol in TCR nanocluster formation has been demonstrated in Tn cells with an experimentally increased cholesterol content. A knock-out of CD4+ Tn cells for the ABCG1 transporter resulted in less cholesterol efflux, which increased the intracellular cholesterol content. Subsequently, more lipid rafts were formed on the plasma membrane, which increased TCR signalling upon activation(Armstrong et al., 2010). All four examples provide strong evidence for the contribution of cholesterol to the formation of TCR nanoclusters, thereby determining the avidity of TCR-antigen interactions.

3.3 Priming of signalling molecules

Cholesterol not only contributes to the exit from quiescence by its role in TCR nanocluster formation, but also by priming intracellular signalling molecules involved in T cell activation. This was demonstrated both in conventional αβ CD4+ T cells and in γδ T cells. In αβ CD4+ T cells, an increased cholesterol content caused by ABCG1 knockout induced increased phosphorylation of extracellular signal-regulated kinase (ERK)1/2, a key downstream effector of TCR signalling. Furthermore, zeta-

chain-associated protein kinase (ZAP)70, a molecule involved in early signalling after TCR activation, was increasingly phosphorylated. Increased ZAP70 and ERK1/2 phosphorylation in Tn cells strengthened TCR signalling upon activation(Armstrong et al., 2010). In γδ T cells, the high baseline cholesterol levels induce phosphorylation of ERK1/2. Upon TCR activation, ERK1/2 signalling is enhanced due to its phosphorylation, which leads to an increased TCR signalling compared to T cells without baseline ERK1/2 phosphorylation(Cheng et al., 2013). By the priming of signalling molecules, intracellular cholesterol levels contribute to the exit from quiescence in Tn cell upon TCR activation.

3.4 Paradoxical effects of cholesterol

Cholesterol on the one hand stabilizes resting TCRs, which maintains Tn cells quiescent. On the other hand, cholesterol contributes to the formation of TCR nanoclusters and to the priming of intracellular signalling molecules, which stimulates Tn cell activation upon interaction with antigens. These functions of cholesterol are seemingly opposing: the stabilization of resting TCRs decreases MHC-antigen avidity, while TCR nanoclustering increases this avidity. However, cholesterol depletion only has a small impact on the percentage of TCRs in the resting state(Swamy et al., 2016), while nanoclustering becomes largely impaired(Molnár et al., 2012).

Furthermore, many examples illustrate the influence of intracellular cholesterol levels on TCR nanoclustering and the subsequent altered activation threshold(Armstrong et al., 2010; Brumeanu et al., 2007; Cheng et al., 2013). In these examples, it could be seen that increased cholesterol levels lead, by enhancing TCR nanoclustering, to an increased sensitivity for T cell activation. This suggests that cholesterol-dependent TCR nanoclustering predominates in defining MHC-antigen avidity. Nevertheless, both resting TCR stabilization and TCR nanocluster formation by cholesterol might be required for a balanced T cell activation, meaning that T cells become activated when needed.

In conclusion, by the stabilization of inactive TCRs, cholesterol prevents unwanted TCR activation in resting T cells. By the formation of nanoclusters and the priming of signalling molecules, cholesterol contributes to TCR activation upon antigen recognition. TCR nanocluster formation is more sensitive to alterations in intracellular cholesterol levels than cholesterol-induced TCR stabilization.

4. Functions of cholesterol in T cell activation and proliferation

T cells become activated upon interaction with their cognate antigen, presented by MHC, in combination with co-stimulatory molecules and cytokines. This leads to T cell proliferation, which is needed for an effective immune response. Therefore, the demand for new cellular components increases upon T cell activation. To meet this need, aerobic glycolysis heavily increases. The switch to a high glycolytic rate is important to sustain ATP generation, while lipids and amino acids can be used for biomass generation(Bietz et al., 2017). Furthermore, glucose itself serves as a substrate for several macronutrients(MacIver et al., 2008). Both ATP and biomass are essential for proliferation and T cell effector functions(Bietz et al., 2017).

Since cholesterol is a major component of plasma membranes it is not surprising that intracellular cholesterol levels rapidly increase upon T cell activation(Bietz et al., 2017; Geltink et al., 2018). Indeed, it has been shown that without an increased cholesterol content a cell cannot make the transition from the G1 to S phase of the cell cycle(Singh et al., 2013).

4.1 Intracellular cholesterol levels: positive feedback

Upon T cell activation, many signalling processes come into effect which heighten intracellular cholesterol levels. One of the changes upon T cell activation is induction of the transcription factor SREBP2. SREBP2 is likely stimulated by a rapidly decreased ER cholesterol content upon activation(Bensinger et al., 2008), as cholesterol is then transported to the plasma membrane to aid in cellular proliferation. Decreased ER cholesterol levels liberate SREBP2 from SCAP. Subsequently, SREBP2 transcribes genes involved in cholesterol biosynthesis and cholesterol uptake, which increases the intracellular cholesterol content (section 2). It has been shown that the induction of SREBP2 is essential for proliferation after activation in human and murine CD4+ and CD8+ T cells(Bensinger et al., 2008; Hu et al., 2015; Kidani et al., 2013).

The increased cholesterol levels induced by SREBP2 not only aid in T cell proliferation by being membrane building blocks, but possibly also by altering cytokine secretion. In mice, treatment with the cholesterol precursor squalene heightened the intracellular cholesterol levels, which was paralleled by increased IL-2 secretion. IL-2 is an autocrine growth factor for T cells, so IL-2 secretion presumably accounts for an increased T cell proliferation rate(Surls et al., 2012).

In addition to SREBP2 induction, stimulation of the TCR inhibits the LXR pathway. LXR inhibition is caused by activation of the enzyme SULT2B1 upon TCR stimulation. By adding sulphate groups to oxysterols, SULT2B1 renders oxysterols unavailable as LXR ligands. Inhibition of the LXR pathway reduces transcription of ABCA1 and ABCG1 genes(Bensinger et al., 2008). The subsequent reduced cholesterol efflux increases intracellular cholesterol levels, although this effect may be limited, because SULT2B1 mediates the formation of cholesterol sulphate from cholesterol(F. Wang et al., 2016). Nevertheless, LXR suppression is essential for human and murine T cell proliferation upon activation(Bensinger et al., 2008).

4.2 Intracellular cholesterol levels: negative feedback

Upon T cell activation, several negative feedback mechanisms become active too. Negative feedback on the cholesterol-increasing pathways is presumably important to prevent excessive immune responses. Until now, two inhibitory loops upon T cell activation have been identified:

An upregulated expression of the transcription factor retinoic acid receptor-related orphan receptor α (RORα) after CD8+ T cell activation is responsible for downregulation of genes involved in cholesterol synthesis, thereby inhibiting CD8+ proliferation in mice. The negative feedback provided by RORα has limited effects, because RORα deficiency only enhances proliferation, while effector functions remain unaffected(Cai et al., 2021). Possibly, this inhibitory regulation mechanism is also

involved in CD4+ T cells. It was seen that in T helper (Th)17 cells, *in vitro* treatment with cholesterol sulphate upregulated RORα, which attenuated inflammatory Th17 signalling(Park et al., 2019). More research is needed to determine whether RORα is also upregulated without experimental intervention, and if this holds true for all CD4+ T cells.

Additional inhibitory feedback is provided by ACAT-1. After CD8+ T cell activation, ACAT-1 expression increases. Since ACAT-1 is involved in cholesterol esterification, the upregulation decreases unesterified cholesterol levels. This limits CD8+ T cell proliferation and effector functions in humans(Li et al., 2018). On the other hand, the inhibitory feedback by ACAT-1 is not observed in CD4+ T cells(Yang et al., 2016).

A third inhibitory feedback loop may also come into effect after a substantial increase in intracellular cholesterol levels upon T cell activation, namely LXR activation. While TCR stimulation inhibits LXR signalling up to 24 hours(Bensinger et al., 2008), maintained increased cholesterol levels will presumably overcome this inhibition and induce LXR to restore cholesterol homeostasis(Bovenga et al., 2015). It would be interesting to monitor LXR signalling over a longer period of time upon TCR activation.

In summary, SREBP2 induction and LXR inhibition upon T cell activation induce increased intracellular cholesterol levels, which are needed for T cell proliferation. An excessive increase in cholesterol content is prevented by negative feedback loops, thus preventing an overactive immune response.

5. Functions of cholesterol in T cell differentiation

After T cell activation and during proliferation, T cells differentiate into a specific T cell subset, defined by costimulatory signalling and the characteristics of the pathogen to which they respond. Infection of host cells with viruses induces the differentiation of CD8+ T cells into effector cytotoxic T lymphocytes (CTLs) to kill the infected cells. On the other hand, CD4+ T cells can differentiate into different types of effector cells. Most well-known are the Th1, Th2, and Th17 cells. Th1 cells generally respond to intracellular pathogens, Th2 to extracellular parasites, and Th17 to extracellular bacteria. Most of these effector T cells move to the side of infection to assist cells of the innate immune system with the elimination of pathogens. Another T cell subset is the T follicular helper (Tfh) cell, which remains in the lymphoid organs to sustain B cells with antibody production. Furthermore, CD4+ T cell differentiation into regulatory T (Treg) cells is important to prevent harmful immune responses. Tregs inhibit effector T cells, thus contributing to a balanced immune response, in which autoimmunity is prevented but harmful pathogens are eliminated. The activation of CD4+ and CD8+ T cells also yields long-lived memory T (Tm) cells, which are important to respond rapidly to a second encounter with the pathogen(Mahnke et al., 2013; Zhu, 2018).

All effector T cells need, to a greater or lesser extent, glycolysis to provide substrates for oxidative phosphorylation. T cells which are less metabolically active, such as Tm, Treg, and Tfh cells, display a repressed glycolytic rate and use fatty acid oxidation to sustain their functions. In addition to this division, every type of T cell relies on a different composition of metabolic pathways. The balance between the different metabolic pathways determines T cell differentiation and functioning, which is extensively reviewed before(Geltink et al., 2018).

The absence or presence of metabolic molecules is, at least partially, caused by asymmetric T cell division in the proliferation stage(Geltink et al., 2018). Prolonged interaction between the T cell and antigen-presenting cell is responsible for asymmetric T cell division. This asymmetry unequally distributes metabolic molecules between the cells, which will up- or downregulate the activity of metabolic pathways, thus influencing T cell differentiation(Chang et al., 2007). Asymmetric T cell division also affects the pathways involved in cholesterol homeostasis. For example, mTOR is unequally distributed between the two daughter cells of an activated CD8+ Tn cell. The cell with a high amount of mTOR is destined to become an effector T cell, while the other will become a Tm cell(Pollizzi et al., 2016). mTOR is, among others, a regulator of cholesterol synthesis(K. L. Ma et al., 2013) and thus, the asymmetric mTOR distribution affects cholesterol homeostasis.

5.1 T helper 1 cells

Signalling molecules involved in cholesterol homeostasis are essential for T cell differentiation and functioning. The influence of cholesterol has been demonstrated in several T cell types. For example, cholesterol biosynthesis regulates Th1 differentiation, functioning, and proliferation. When the cholesterol content in Tn cells in mice was increased by the administration of the cholesterol precursor squalene, signal transducers and activators of transcription (STAT)4 and STAT5 became phosphorylated. Upon activation, these STAT4/STAT5-primed Tn cells differentiated into Th1 effector cells(Surls et al., 2012).

The activity of the cholesterol biosynthesis pathway also influences the cytokine expression profile of Th1 cells. The Th1 response to a pathogen must be restricted to prevent an overactive immune response causing tissue damage. Therefore, it is of vital importance that a part of the generated Th1 cells start to express the anti-inflammatory cytokine IL-10, instead of the inflammatory cytokine interferon gamma (IFNγ). Perucha et al. saw that inhibition of the cholesterol biosynthesis pathway by statins or by the oxysterol 25-hydroxycholesterol (25-HC) in human cell culture prevents the shift from IFNγ to IL-10 expression. Thus, they suggest that a flux of the cholesterol biosynthesis pathway is essential for the induction of IL-10 expression in Th1 cells. This knowledge may even explain why

the use of the HMGCR-inhibiting statins increases the risk of inflammatory disorders such as rheumatoid arthritis: the inhibition of a switch to IL-10 expression by statins could lead to overactive Th1-mediated immune responses(Perucha et al., 2019).

The cholesterol biosynthesis pathway also generates isoprenoids which could modify proteins and thereby alter protein activity(Grünler et al., 1994). It was demonstrated that the isoprenoid geranylgeranyl-pyrophosphate (GGPP) mediates Th1 proliferation, while both GGPP and its precursor farnesyl-PP regulate Th1 differentiation(Dunn et al., 2006). This way, not only cholesterol levels themselves, but also the cholesterol pathway intermediates are involved in Th1 differentiation and functioning.

5.2 T helper 2 cells

For Th2 differentiation, several studies imply that it is important that cholesterol biosynthesis is suppressed. In a rat model of autoimmune myocarditis, it was demonstrated that the HMGCR inhibitor atorvastatin stimulated Th2 differentiation, while Th1 differentiation was inhibited(Liu et al., 2005). Similar results were obtained in a mouse model of autoimmune encephalomyelitis. In this model, atorvastatin also shifted the Th1/Th2 balance towards Th2 differentiation(Youssef et al., 2002). Dunn and colleagues elucidated the underlying mechanism of the Th2-stimulating effects of atorvastatin. Namely, inhibition of the cholesterol biosynthesis pathway reduces the generation of isoprenoids. Atorvastatin-mediated reduction of the isoprenoids GGPP and farnesyl-PP, which determine Th1 differentiation, induces a Th2 bias(Dunn et al., 2006).

In contrast with the examples described above, it was seen that in an eosinophilic asthma mouse model, type 2 cytokine secretion was promoted by LXR activity(Smet et al., 2016). LXR negatively regulates intracellular cholesterol levels by promoting efflux, but not by inhibiting cholesterol biosynthesis(Bovenga et al., 2015). Therefore, the experiments by Smet et al. imply that the intracellular cholesterol levels, but not cholesterol biosynthesis intermediates, are important in setting the Th1/Th2 balance.

The seemingly contradicting findings of Smet et al. compared to that of Liu et al., Youssef et al., and Dunn et al. could be explained by differences in animal models. Whereas Dunn et al. performed experiments with mice having a baseline Th2 bias, the other researchers used animals having a baseline Th1 bias(Dunn et al., 2006; Liu et al., 2005; Smet et al., 2016; Youssef et al., 2002). Furthermore, in the eosinophilic asthma mouse model, the effects of atorvastatin have not been studied. Therefore, it remains a possibility that both cholesterol itself and products of the cholesterol pathway are involved in the regulation of Th2 differentiation. More studies into different experimental models would be useful to elucidate the paradoxical results.

5.3 T helper 17 and γδT cells

Cholesterol biosynthesis activates the transcription factor RORγt, which is responsible for the expression of Th17-specific cytokines. Increased cholesterol biosynthesis is therefore essential for Th17 differentiation. Cholesterol biosynthesis induces RORγt in at least two ways: the cholesterol precursor desmosterol is a potent RORγt ligand, and cholesterol sulphates derived from cholesterol strongly induce RORγt. Cholesterol sulphates are also ligands for the transcription factor LXR, responsible for the transcription of genes which stimulate cholesterol efflux(Hu et al., 2015). Indeed, it has been shown that LXR activation inhibits Th17 differentiation and expansion in mice and humans(Cui et al., 2011; Parigi et al., 2021). However, in differentiating Th17 cells cholesterol sulphate favours RORγt stimulation over LXR stimulation. This is crucial to maintain high intracellular cholesterol levels, thus contributing to Th17 differentiation(Hu et al., 2015). The cholesterol-induced RORγt activation may also play a role in γδT cells, since inhibition of cholesterol synthesis decreased IL-17A production in γδT cells(Hu et al., 2015).

It is striking that fatty acid synthesis is also required for proper functioning of Th17 cells(Geltink et al., 2018). It is known that the SCAP-regulated SREBP-1a isoform controls both cholesterol and fatty acid biosynthesis(Eberlé et al., 2004). The role of the different SREBP isoforms should therefore be considered in future research on Th17 cells.

5.4 Regulatory T cells

Differentiation of activated Tn cells into Treg cells likely relies on inhibition of cholesterol synthesis. It was seen that activation of mTOR-deficient Tn cells shifted the Treg/Teffector balance towards Treg cells in mice(Delgoffe et al., 2009). Since mTOR stimulates SREBP2-dependent cholesterol synthesis(K. L. Ma et al., 2013), differentiation of CD4+ T cells into Treg cells might require a reduced cholesterol synthesis pathway. A reduced cholesterol biosynthesis is possibly caused by intracellular cholesterol accumulation. Indeed, LDLR knock-out mice with additional decreased expression of the cholesterol transporter ABCG1 showed an increased intracellular cholesterol accumulation and subsequent downregulation of mTOR in Tn cells. Tn cells with a lower activity of the mTOR pathway favoured differentiation into Treg cells(Cheng et al., 2016).

In contrast to the differentiation of Tn cells into Tregs, the functioning of reactivated Treg cells relies on cholesterol synthesis. Upon stimulation of already differentiated Tregs, mTORC1 and liver kinase B1 (LKB1) become activated. Both proteins independently induce the cholesterol biosynthesis pathway. Upregulation of the cholesterol biosynthesis pathway stimulates Treg proliferation and functioning by upregulation of the suppressive proteins cytotoxic T-lymphocyte associated protein 4 (CTLA4) and inducible T cell costimulator (ICOS)(Timilshina et al., 2019; Zeng et al., 2013). Furthermore, the cholesterol biosynthesis pathway generates the isoprenoid GGPP, which maintains Treg function by the phosphorylation of STAT5. STAT5 induces IL-2 signalling, which is important for Treg survival(Timilshina et al., 2019).

5.5 Follicular T helper cells

Tfh cells require mTOR for differentiation and functioning, both under unstimulated conditions and upon viral infections(Xu et al., 2017; Zeng et al., 2016). mTOR regulates several metabolic pathways, one of them being the SREBP2-dependent cholesterol biosynthesis(K. L. Ma et al., 2013). Therefore, differentiation of Tfh cells might require increased cholesterol levels or intermediates of the cholesterol biosynthesis pathway.

However, it has been observed that when mTOR is stimulated by IL-2, an interleukin with an important role in proliferation, a shift from Tfh to Th1 differentiation is induced(Ray et al., 2015). Possibly, several other pathways determined by IL-2 and by mTOR influence T cell differentiation. Namely, mTOR regulates T cell differentiation in various ways, as has been recently reviewed by Wang and colleagues(P. Wang et al., 2020).

5.6 Cytotoxic T lymphocytes

For activated CD8+ T cells to become CTLs, increased cholesterol levels are essential. It was seen that after activation, CD8+ T cells displayed enhanced cholesterol synthesis which potentiated their effector functions. Moreover, inhibition of the esterification enzyme ACAT1 markedly increased CTL killing activity in mice, which was caused by increased cholesterol levels(Yang et al., 2016). It is unknown if intermediates of the cholesterol biosynthesis pathway also influence CTLs.

5.7 Memory T cells

To differentiate into Tm cells, CD8+ T cells might require a suppressed cholesterol biosynthesis pathway. Indeed, mTOR inhibition by rapamycin increased CD8+ Tm cell numbers in mice(Kidani et al., 2013). Since mTOR stimulates SREBP2-dependent cholesterol biosynthesis(K. L. Ma et al., 2013),

inhibition of the cholesterol biosynthesis pathway could be important for CD8+ Tm cells. It would be interesting to further investigate the activity of the cholesterol pathways in CD8+ Tm cells.

In contrast to CD8+ Tm cells, CD4+ Tm cells possibly require cholesterol biosynthesis for differentiation and functioning. In humans and mice with inflammatory bowel disease, namely, microbiota-specific CD4+ Tm cells were reduced and impaired by mTOR inhibition(Q. Zhao et al., 2020).

Indeed, there are many differences between CD4+ and CD8+ Tm cells. For example, CD8+ Tm cells tend to respond more rapidly upon an encounter with cognate antigens compared to CD4+ Tm cells(Seder & Ahmed, 2003). Future research should explore whether the differences in CD4+ and CD8+ Tm cell functioning are caused by the differences in cholesterol biosynthesis activity.

5.8 Conclusion

The cholesterol pathway is involved the establishment and maintenance of different T cell subsets. In summary, Th1, Th17, γδT, and CTL cells require high cholesterol levels or an active cholesterol biosynthesis pathway for their functioning. On the other hand, low cholesterol levels and a low activity of cholesterol biosynthesis is essential for Th2 cells. Tregs display paradoxical effects: while the differentiation of Tregs from Tn cells presumably relies on inhibition of cholesterol biosynthesis, the functioning of reactivated Tregs requires an active flux of the cholesterol biosynthesis pathway. A similar inconsistency is seen in Tm cells: while CD4+ Tm cells might require a high activity of cholesterol biosynthesis, CD8+ Tm cells possibly require a low activity. Research to elucidate the mechanisms responsible for these paradoxes, as well as to the unknown role of cholesterol in Tfh cells, would aid in the understanding of conditions in which cholesterol metabolism is abnormal. So far, research mainly focussed on the activity of the cholesterol biosynthesis pathway. It would be interesting to also unravel the expression of the LDLR and the ABCA1/G1 transporters to get a full overview of the regulation cholesterol homeostasis in T cell differentiation.

6. Defective cholesterol metabolism and T cell function

Proper cholesterol metabolism is essential for the functioning of T cells in the resting, activation, proliferation, and differentiation stage. When cholesterol homeostasis becomes disturbed, several diseases may occur. Depending on the cause, defects in cholesterol metabolism could lead to cardiovascular, neurological, and growth impairments. Disturbed cholesterol homeostasis can be caused by lifestyle or by mutations, although a combination of both is also possible. In this section, the effects of hypercholesterolemia, either caused by lifestyle or by mutations, on T cells will be discussed. Next, two diseases in which cholesterol export or cholesterol biosynthesis is disturbed will be reviewed.

6.1 Diet-induced hypercholesterolemia

A diet containing many saturated fatty acids and cholesterol, also called a Western diet, could lead to hypercholesterolemia: high plasma cholesterol levels. Especially high LDL-cholesterol levels (> 4.13 mmol/L) are correlated with several diseases, while high HDL-cholesterol levels are positively associated with disease outcome(Mannu et al., 2013). The most well-known disease caused by hypercholesterolemia is atherosclerosis. In atherosclerosis, T cells have several proatherogenic as well as atheroprotective roles. For example, while Th1 and Tfh cells are proatherogenic, Tregs are atheroprotective, and Th2 and Th17 cells are able to exert both functions(Saigusa et al., 2020). Next to atherosclerosis, there is increasing evidence that hypercholesterolemia contributes to the development of Alzheimer's disease and cancer(Gamba et al., 2012; Luo et al., 2020). Understanding how changes in cholesterol metabolism could affect T cell functioning is therefore of significant relevance.

Intracellular cholesterol levels

In diet-induced hypercholesterolemia, the high number of LDL particles in the blood plasma presumably results in an increased uptake of LDL by the LDLR. In wild type (WT) T cells which were exposed to exogenous cholesterol, it was seen that the intracellular cholesterol content increased within four hours(Cheng et al., 2013; Mailer et al., 2017b). In theory, increased intracellular cholesterol levels will lead to a decreased cholesterol biosynthesis and a reduced LDLR expression, while cholesterol esterification and efflux will increase (section 2). These compensatory mechanisms might indeed come into effect at first, but may be exhausted upon sustained hypercholesterolemia. It remains to be understood how the activity of compensatory mechanisms to maintain cholesterol homeostasis changes during the development of diet-induced hypercholesterolemia.

Effects on resting and activated T cells

Hypercholesterolemia affects T cells in the resting and activation/proliferation stage. When mice were injected with the cholesterol-precursor squalene, the number of resting CD4+ T cells was increased(Surls et al., 2012). Furthermore, *in vitro* incubation of resting WT T cells with exogenous cholesterol increased the sensitivity towards TCR stimulation, which was caused by heightened intracellular cholesterol levels. The incubated T cells also increasingly proliferated upon activation(Cheng et al., 2013; Mailer et al., 2017b).

These observations are in line with the established roles of cholesterol in resting and activated T cells: an increased cholesterol content will increase TCR nanoclusters and prime molecules involved in activation, thus increasing sensitivity for activation (section 3). Furthermore, since cholesterol is essential for cellular proliferation, the increased cholesterol levels will aid in proliferation after activation (section 4).

Indeed, it seems that intracellular cholesterol accumulation leads to autoimmune responses. In a large cohort study, low HDL-cholesterol levels were associated with a higher risk of autoimmunity(Madsen et al., 2019). Low HDL-cholesterol levels reflect an impaired reverse

cholesterol transport from peripheral cells to the liver. Therefore, low HDL-cholesterol levels indicate the accumulation of cholesterol in T cells and other peripheral cells(Ouimet et al., 2019). Furthermore, several studies indicate that hypercholesterolemia exacerbates autoimmune diseases, such as rheumatoid arthritis and psoriasis, by overactive T cells(Ryu et al., 2019).

Effects on T cell differentiation

Diet-induced hypercholesterolemia alters T cell differentiation and functions *in vivo*. In hypercholesterolemic WT mice, augmented numbers of thymic and peripheral Treg cells were observed. This increase is presumably caused by an increased TCR signalling, because Treg proliferation depends on homeostatic TCR signalling(Mailer et al., 2017b). It would be expected that an increased Treg number reduces the amount of effector T cells. However, the opposite is true: the number of effector T cells is increased in hypercholesterolemic WT mice. Thus, it seems that the inhibitory actions of Tregs on effector T cells are diminished in hypercholesterolemia(Mailer et al., 2017b).

Unfortunately, much remains unclear about the effects of diet-induced hypercholesterolemia on Th1, Th2, and Th17 cells. Since cholesterol stimulates Th1 and Th17 differentiation, an increased number would be expected (section 5). Indeed, intrahepatic Th17 numbers became increased in WT hypercholesterolemic mice. However, intrasplenic Th17 numbers remained unaltered. Furthermore, Th1 numbers in the liver were not changed by diet-induced hypercholesterolemia, but no information is available about Th1 numbers in other organs(Mailer et al., 2017a).

Concerning CTLs, it is established that increased cholesterol levels are required for their effector functions (section 5). It could therefore be postulated that hypercholesterolemia would increase CTL-mediated killing. Indeed, it appears that obese cancer patients respond better to several types of cancer therapy compared to lean patients(Turbitt et al., 2020). The underlying mechanisms of this clinical finding are not identified yet, but hypercholesterolemia influencing CTLs may play a role. On the other hand, it has been observed that high extracellular cholesterol levels in a tumor microenvironment induce ER-stress in CTLs, thereby impairing their effector function(X. Ma et al., 2019). These contrasting studies affirm that hypercholesterolemia is a systemic disease, affecting the body in many ways. To understand clinical outcomes in hypercholesterolemic patients, research into the underlying mechanisms of hypercholesterolemia on the different T cell subsets is of great importance.

Future research

Because hypotheses based on the known roles of cholesterol in the differentiation stage of T cells not always hold true, it is of high interest to investigate the changes in T cell subsets during the development of diet-induced hypercholesterolemia further. Several factors should be considered.

Firstly, influences of hypercholesterolemia on T cells could be location-specific: results differ between Th17 cells from spleen and liver(Mailer et al., 2017a). These results indicate that other factors next to cholesterol levels influence T cells. For example, hypercholesterolemia could affect other cell types, which then influence T cells. In this way, the presence or absence of these cell types induces location-specific effects of hypercholesterolemia on T cells.

Secondly, LDL particles are not composed of cholesterol alone, but also contain many proteins, phospholipids, triglycerides, and other fatty acids. Therefore, the increased uptake of LDL particles in diet-induced hypercholesterolemia may exert additional effects on T cells by the other components. For example, an increased number of fatty acids is known to alter lipid metabolism. The influences of lipid metabolism on T cell functions is reviewed by Lochner and colleagues(Lochner et al., 2015).

Thirdly, the modification of LDL particles should be considered. The prolonged presence of many LDL particles in the circulation allows for their oxidation, which generates oxidated LDL (oxLDL) particles. T cells are able to endocytose oxLDL, which modulates T cell functions. oxLDL is a major contributor to inflammation. Additional information about oxLDL is available in a review by Rhoads and Major(Rhoads & Major, 2018).

Fourthly, it is worthwhile to study T cell and cytokine profiles in serum of human patients with diet-induced hypercholesterolemia. This would aid in the translation of results obtained in mouse models to humans. Furthermore, measurements of T cell and cytokine levels in serum would provide information about the effects of a therapy. Currently, hypercholesterolemic patients are treated with the HMGCR-inhibiting statins according to the clinical standards. The efficacy of statin treatment is monitored by measurements of LDL and HDL levels(Levinson, 2020). In addition to these parameters, T cell and cytokine profiles in serum might be a useful measure of therapy monitoring.

In conclusion, diet-induced hypercholesterolemia affects T cells in many ways, which contributes to the development of diseases. However, much is still unknown. Since the prevalence of diet-induced hypercholesterolemia is increasing(Virani et al., 2021), it is important to conduct more research to the changes in T cells upon high plasma cholesterol levels. This may aid in the development of treatment and prevention methods.

6.2 Familial hypercholesterolemia

Hypercholesterolemia caused by genetic mutations is called familial hypercholesterolemia (FH). FH is an autosomal dominant disease with a relatively high prevalence, estimations range from 1:500 to 1:100. In most FH patients, genes coding for the LDLR or apolipoprotein B (Apo-B), which is the principal component of an LDL particle, are affected. Both LDLR and Apo-B mutations result in a decreased clearance of LDL from the circulation, which heightens the plasma LDL levels(Bouhairie & Goldberg, 2015). Similar to diet-induced hypercholesterolemia, FH contributes to the development of many diseases, of which cardiovascular events are the most prevalent(Bouhairie & Goldberg, 2015).

Intracellular cholesterol levels

Experiments in human fibroblasts derived from FH patients showed that a reduced uptake of LDL dysregulates intracellular cholesterol biosynthesis. Normally, a constitutive uptake of LDL by LDLR has a braking effect on cholesterol biosynthesis. Mutations in FH remove this brake, because LDL uptake is greatly reduced. In this way, HMGCR activity is upregulated in FH, which increases cholesterol biosynthesis. The heightened flux of the cholesterol pathway and the subsequent increased net sum of intracellular cholesterol levels alter cellular functioning(Goldstein & Brown, 1973; Suárez-Rivero et al., 2018).

It is still unknown if the *in vitro* observations in human fibroblasts of FH patients apply to T cells as well. Although the mechanisms of cholesterol biosynthesis, uptake, esterification, and efflux are present in T cells, the relative contribution of these four pathways in T cells is lacking (section 2). In any case, the results of Goldstein and Brown and Suárez-Rivero et al. does not exclude the possibility that intracellular cholesterol levels in T cells of FH patients might be increased too(Goldstein & Brown, 1973; Suárez-Rivero et al., 2018).

As it appears, cholesterol metabolism is differentially dysregulated in FH compared to diet-induced hypercholesterolemia. In diet-induced hypercholesterolemia the LDLR is not affected, and intracellular cholesterol levels are increased by an increased uptake of cholesterol (Cheng et al., 2013; Luo et al., 2020; Mailer et al., 2017b). In FH however, intracellular cholesterol levels are possibly increased by a positive feedback loop, because of the reduced cholesterol uptake(Goldstein & Brown, 1973; Suárez-Rivero et al., 2018). Therefore, the source of cholesterol may differ for the

cells in diet-induced hypercholesterolemia and FH. In some yet unknown ways, this may affect T cells.

Effects on T cells

Most data on T cells in FH comes from mouse models. The most frequently used mouse models to study FH are Ldlr-/- mice and Apoe-/- mice, which are knocked-out for LDLR and Apolipoprotein E (Apo-E), respectively. Apo-E is part of chylomicrons and several lipoprotein particles. Both mice models develop severe hypercholesterolemia and are seen to be the most representable animal models for human FH(Lo Sasso et al., 2016; Pendse et al., 2009).

Based on the known cholesterol functions in the different stages of T cells, increased cholesterol biosynthesis will hypothetically lead to increased TCR sensitivity and T cell proliferation (section 3 and 4). An enhanced TCR sensitivity and cellular proliferation upon stimulation is indeed observed in Ldlr-/- hypercholesterolemic mice(Mailer et al., 2017b). The differentiation of T cells into Th1, Th17, and CTL will probably increase in FH, while Th2 and Treg will decrease (section 5). Indeed, the number of Th1 cells in the liver was increased in Ldlr-/- mice compared to WT mice, which was even stronger when Ldlr-/- mice were fed with a Western diet(Mailer et al., 2017a). Furthermore, in Ldlr-/- mice with an additional mutation in an ApoB mRNA editing enzyme (LDb mice), the number of splenic Th1 cells was increased(Lim et al., 2014).

However, severe hypercholesterolemia in Apoe-/- mice induced a switch from splenic Th1 to Th2 cells. These Th2 cells displayed an immune reaction against oxLDL(Robertson et al., 2004; Zhou et al., 1998). It has been speculated that very high concentrations of lipids and/or lipoproteins can induce a shift to Th2 by binding to nuclear receptors, such as peroxisome proliferator-activated receptor (PPAR)-γ which inhibits Th1 differentiation(Robertson et al., 2004). The Th1 to Th2 shift in severe hypercholesterolemia might explain the association between hypercholesterolemia and allergy, which is observed in some studies(Manti et al., 2017).

Regarding Th17 cells, hypercholesterolemia in LDb mice was associated with increased serum IL-17 levels as well as increased numbers of Th17 cells in lymphoid tissues(Lim et al., 2014). However, Mailer and colleagues found that the number of Th17 cells in the liver remains unaltered in hypercholesterolemic Ldlr-/- mice. Unfortunately, the Th17 cells in the spleen of Ldlr-/- mice were not assessed(Mailer et al., 2017a). Probably, the influence of hypercholesterolemia on Th17 cells is location-specific. The liver is, namely, a differentially regulated lymphoid organ in which many cell types influence each other. For example, the hepatocytes themselves could function as antigen-presenting cells(Crispe, 2009). In this way, the T cell profile in the liver may differ from that in other lymphoid organs.

The changes in Treg cells in FH are varying. In Ldlr-/- mice, splenic and circulating Treg numbers initially increased, but sustained hypercholesterolemia eventually decreased Treg numbers, which corresponded with an increase in effector T cells(Maganto-García et al., 2011). In LDb mice, the Treg content was relatively higher in secondary lymphoid organs and the thymus compared to WT mice(Lim et al., 2014). Furthermore, Mailer et al. found that the intrahepatic Treg content linearly increased with cholesterol intake in Ldlr-/- mice(Mailer et al., 2017a). On the other hand, hypercholesterolemic Apoe-/- mice displayed a conversion of lymphoid Treg cells to Tfh cells. This conversion was caused by intracellular cholesterol accumulation, which attenuated IL-2 signalling. The IL-2 signalling pathway stabilizes Treg cells and inhibits Tfh cells(Gaddis et al., 2018). Altogether, these results indicate that in FH in mice there is an initial increase in Treg cells as a compensatory mechanism to several inflammatory stimuli. This compensatory mechanism eventually fails, seen by decreased Treg numbers, Treg conversion to Tfh, and decreased suppression of effector T cells.

The function of CTLs is reduced in FH, as observed in both Apoe-/- and Ldlr-/- mice. Hypercholesterolemia impaired CTL cytotoxicity and IFN-γ production. Moreover, less CTLs were recruited in the circulation of virus-infected mice. Ludewig et al. speculate that the impaired CTL response is caused by impaired signalling pathways by hypercholesterolemia(Ludewig et al., 2001). It is striking that the response of CTLs to hypercholesterolemia is the opposite from the known functions of cholesterol in CTLs, namely that high intracellular cholesterol levels are essential for a proper CTL response(Yang et al., 2016). It is therefore very likely that other factors in hypercholesterolemia influence the response of T cells to altered cholesterol levels.

Future perspective

Similar to diet-induced hypercholesterolemia, FH influences the functions of T cells in various manners. Although the changes in T cells have been unravelled more for FH than for diet-induced hypercholesterolemia, much is still unknown. The biggest impairment in the research to T cells in FH is the lack of research in humans. Unfortunately, results in animal models are not always similar to results in humans(Y. Zhao et al., 2020). It is therefore important that future research considers whether the results obtained in mice also apply for humans.
Currently, FH patients are treated with statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, the latter preventing the PCSK9-mediated LDLR degeneration to increase LDLR expression(Santos, 2019). The combination of statin or PCSK9 treatment and impaired LDL uptake on T cells would be of high interest to study.

6.3 Tangier disease

Another disease in which cholesterol metabolism is defective is Tangier disease. Tangier disease is a rare disease, caused by mutations in the ABCA1 gene. The subsequent reduced expression or functioning of the ABCA1 transporter prevents cholesterol efflux to nascent HDL particles. This results in low plasma HDL-cholesterol levels and intracellular cholesterol accumulation. Tangier disease manifests itself in a range of clinical symptoms which depend on the specific mutation. Symptoms include neuropathy, anaemia, splenomegaly, and atherosclerosis(Hooper et al., 2020).

In Tangier disease patients, serum cytokine concentrations are increased, which has been associated with increased inflammation of atherosclerotic plaques(Bochem et al., 2015). Unfortunately, only the effects of the cytokine levels on macrophages have been researched(Bi et al., 2015; Bochem et al., 2015). Since the ABCA1 transporter also plays a role in T cells, these immune cells will likely be affected too in Tangier disease.

Hypothesized T cell alterations

It can be hypothesized that the intracellular cholesterol accumulation in T cells will increase the plasma membrane cholesterol content. This will increase both the sensitivity of T cells for activation and the subsequent proliferation (section 3 and 4). Indeed, in mice knocked out for an other cholesterol transporter, ABCG1, increased plasma membrane cholesterol and increased sensitivity and proliferation of T cells were observed(Armstrong et al., 2010). Increased T cell proliferation could contribute to the observed splenomegaly in Tangier patients(Bochem et al., 2015).

The intracellular cholesterol accumulation will undoubtedly alter T cell differentiation. As seen in the previous paragraphs about diet-induced and familial hypercholesterolemia, it is not easy to predict the exact T cell subsets outcome. Besides, cholesterol biosynthesis might be reduced in Tangier disease as a compensatory mechanism for the intracellular cholesterol accumulation. T cell differentiation would be changed by altered cholesterol biosynthesis as well. Furthermore, many other factors next to cholesterol play a role in T cell differentiation(Zhu, 2018). For example, cytokines, such as IL-12, secreted by the overactive macrophages could induce Th1 differentiation(Hsieh et al., 1993).

Future perspective
Primary manifestations in Tangier disease, such as atherosclerosis, are currently treated with statins(Hooper et al., 2020). Statins however do no tackle the intrinsic problem, which is the reduced cholesterol efflux. Therefore, more research to an effective therapy in Tangier disease would be worthwhile. Ideally, the mutated ABCA1 gene would be replaced by a healthy copy of the gene. That would be possible with gene therapy(Tang & Xu, 2020). Furthermore, more research into the disease mechanisms of Tangier disease, also addressing T cells, would increase the understanding of this rare disease.

6.4 Smith-Lemli-Opitz syndrome

Mutation-induced defects in cholesterol metabolism could also exist in the cholesterol biosynthesis pathway. The most prevalent disease caused by a cholesterol biosynthesis defect is the Smith-Lemli-Opitz syndrome (SLOS). In SLOS, the 7-dehydrocholesterol reductase (DHCR7) gene is mutated. DHCR7 codes for the enzyme DHCR7, which converts 7-DHC to cholesterol. The mutated DHCR7 gene leads to cholesterol deficiency, especially in the brain that cannot be accessed by diet-obtained cholesterol, and to accumulation of 7-DHC. Clinically, growth abnormalities and mental retardation are observed(DeBarber et al., 2011). Furthermore, food allergies and sudden devastating infections occur relatively frequently in SLOS, indicating abnormal T cell functioning(Kelley & Hennekam, 2000).

Based on the known mechanisms of cholesterol homeostasis (section 2), an increased LDLR expression is expected in SLOS to take up more lipoproteins, thus restoring the cholesterol levels. While Dhcr7-/- mice do not show changes in LDLR mRNA and protein levels compared to WT mice(Fitzky et al., 2001), in a SLOS infant patient a marked increase in LDLR in liver and brain cells was observed(Ness et al., 1997). Furthermore, patients of the disease sitosterolemia, a rare disease in which cholesterol biosynthesis is also reduced, have an increased expression of LDLR in mononuclear leukocytes(Nguyen et al., 1990). These studies indicate that SLOS mouse models do not fully represent SLOS in human patients and results should be translated with care.

Effects on T cells
Both the reduced cholesterol content and accumulated 7-DHC disrupt lipid raft signalling. It was seen in mast cells that 7-DHC is incorporated in lipid rafts and that the altered 7-DHC/cholesterol ratio influenced several signalling processes(Kovarova et al., 2006). Lipid raft signalling in T cells is presumably affected as well. Indeed, it has been observed that the increased 7-DHC/cholesterol ratio altered the properties of the voltage-gated Kv1.3 channel in T cells. This was associated with reduced T cell activation and proliferation(Balajthy et al., 2016).

By a negative feedback loop, cholesterol biosynthesis becomes decreased even more in SLOS. The accumulation of 7-DHC decreases HMGCR levels and activity in Dhcr7-/- mice. In this way, the cholesterol pathway is inhibited still greater(Fitzky et al., 2001). If the negative feedback loop is also present in T cells, it would not only exacerbate disrupted T cell activation and proliferation, but also T cell differentiation. Possibly, Th1, Th17, Treg, and CTL differentiation will be impaired, because cholesterol biosynthesis is essential in these T cell subsets. On the other hand, Th2 differentiation might increase, because the inhibitory effect of the cholesterol biosynthesis pathway on Th2 cells is vanished (section 5).

Corresponding to these hypotheses, allergies are frequently observed in SLOS(Kelley & Hennekam, 2000), which may be caused by an increased number and/or activity of Th2 cells. Furthermore, frequently occurring infections are observed in SLOS(Kelley & Hennekam, 2000). The underlying reason for this could be a disrupted T cell activation, proliferation, and differentiation of Th1, Th17, and Treg. Nevertheless, more research into the T cell activities and subsets is necessary to confirm these hypotheses.

Future perspective

In SLOS, reduced cholesterol levels and 7-DHC accumulation presumably lead to disrupted T cell activation, proliferation, and differentiation. Cholesterol supplementation is a standard intervention to restore intracellular cholesterol levels. In addition, cholesterol supplementation downregulates the cholesterol biosynthesis pathway and thus suppresses accumulation of 7-DHC. However, cholesterol supplementation does not reach the brain and therefore, the beneficial effects are limited(DeBarber et al., 2011). Future research should focus on the development of medicines or supplements which cross the blood-brain-barrier. In this research, it should be kept in mind that SLOS mouse models are not identical to the situation in humans. Potentially, human brain organoids could be a representative study model for SLOS.

7. Discussion

Cholesterol metabolism is critical for innate and adaptive immune cell function, including T-cells. In the current review, the physiological and pathophysiological influences of cholesterol metabolism on T cells in different stages of activation have been described.

7.1 Summary

Cellular cholesterol homeostasis consists of four processes: cholesterol biosynthesis, uptake, esterification, and efflux. Low intracellular cholesterol levels stimulate cholesterol biosynthesis and uptake, while high intracellular cholesterol levels stimulate cholesterol efflux and esterification (section 2). In Tn cells, cholesterol stabilizes resting TCRs, thus preventing unwanted T cell activation. Furthermore, cholesterol contributes to nanocluster formation and primes signalling molecules, which aids in the activation of T cells upon antigen recognition (section 3). After T cell activation, intracellular cholesterol levels increase by several feedback mechanisms, which is essential for T cell proliferation (section 4). In the differentiation stage of T cells, the activity of the cholesterol biosynthesis pathway determines into which T cell subset an activated T cell differentiates. Research indicates that high cholesterol levels or an active cholesterol biosynthesis pathway stimulate differentiation into Th1, Th17, γδT, CTL, and CD4+ Tm cells. A suppressed cholesterol biosynthesis pathway leads to differentiation into Th2 and CD8+ Tm cells. In Tregs, cholesterol plays a dual role: differentiation of Tregs from Tn cells relies on inhibition of cholesterol biosynthesis, while the functioning of reactivated Tregs presumably requires an active flux of the cholesterol biosynthesis pathway (section 5).

Hypercholesterolemia has a high prevalence, affecting 12% of adult Americans(Benjamin et al., 2019), and can be induced by diet or by FH, the latter of which is characterized by mutations in LDLR or ApoB. Both in diet-induced hypercholesterolemia and FH cholesterol levels in the blood stream are increased, which may lead to increased intracellular cholesterol levels as well, thus enhancing T cell activation and proliferation. The effects of hypercholesterolemia on T cell differentiation need more investigation. In two other genetic diseases in which cholesterol metabolism is disrupted, Tangier disease and SLOS, clinical signs indicate that T cells are disrupted in different stages. However, the exact mechanisms have poorly been researched and need further investigation (section 6).

7.2 Outstanding questions

Throughout this review, many suggestions for future research have been given. The most important outstanding questions will be discussed in the current section.

Cholesterol uptake, biosynthesis, efflux, and esterification

Until now, research on cholesterol in T cells has mainly focussed on the cholesterol biosynthesis and cholesterol efflux pathway. However, it remains to be understood to which extent these and the other two pathways involved in cholesterol homeostasis (efflux and esterification) contribute to intracellular cholesterol levels in T cells. Besides, it is unknown how this relative contribution changes during the different stages of activation in T cells. An important outstanding question to be answered is therefore: what is the relative contribution of the four different pathways of cholesterol homeostasis (i.e., uptake, biosynthesis, efflux, and esterification) in T cells in different stages of activation?

A related question is: are cholesterol biosynthesis and lipoprotein uptake redundant mechanisms? It is still unidentified whether the one can compensate for the loss of the other, which is relevant in diseases such as FH and SLOS. In FH lipoprotein uptake is disturbed, while in SLOS cholesterol biosynthesis is affected. Research indicates that in FH cholesterol biosynthesis is increased and in SLOS cholesterol uptake is enhanced (section 6). However, it remains to be understood if these

compensatory mechanisms are also present in T cells and how that affects the intracellular cholesterol content and T cell functions.

Furthermore, while many effects of cholesterol biosynthesis and efflux on T cells have been unravelled, it remains unclear how cholesterol esterification and uptake influence T cell functioning. Cholesterol esterification is known to regulate several pathways, such as signalling complexes controlled by amyloid-β oligomers, the causative agent in Alzheimer's disease(West et al., 2017). The cholesterol esterification pathway also stimulates the Wnt/β-catenin pathway, which is involved in the development of cancer metastases(Lee et al., 2018). Potentially, cholesterol esterification also controls these and other pathways in T cells, thus affecting their functioning. Lipoprotein uptake may also mediate several cellular effects. Upon internalization, lipoproteins are broken down into cholesterol and amino acids. While a number of effects exerted by internalized cholesterol are known (section 2), lipoprotein-derived amino acids may influence signalling pathways too. Unfortunately, literature is scarce on data concerning the effects of lipoprotein-derived amino acids. An outstanding question therefore is: how do cholesterol esterification and uptake influence T cell function?

The answer to these questions will increase the understanding of diseases in which cholesterol metabolism is disrupted, such as hypercholesterolemia, Tangier disease, and SLOS. One of the ways to obtain partial answers is the available dataset on RNA sequencing data in T cells both in resting and activation stages in diverse anatomic compartments(Szabo et al., 2019). Analysis of these data will provide an overview of the transcription rates of the genes coding for the proteins involved in the different pathways of cholesterol homeostasis. RNA sequencing data in normocholesterolemic individuals could be compared with hypercholesterolemic individuals. Another method to investigate the different cholesterol homeostasis pathways is with isotope tracers. By radioactive labelling of, for example lipoproteins or cholesterol, the metabolism of these substances could be traced both *in vitro* and *in vivo*(Chan & Watts, 2006).

Intermediates or cholesterol

Another outstanding question is: when T cells rely on cholesterol biosynthesis, is that caused by effects of intracellular cholesterol itself or by intermediates of the cholesterol biosynthesis pathway? It is known for several T cell subsets that cholesterol biosynthesis is needed for differentiation and functioning. However, for most of them it is not known which underlying factors are responsible for the reliance on cholesterol biosynthesis. That could be either effects of cholesterol intermediates or effects of cholesterol itself. For example, the cholesterol biosynthesis pathway also generates isoprenoids, which can alter protein activity and thereby influence cellular functioning(Grünler et al., 1994) (section 5).

To elucidate whether and which cholesterol intermediates affect cellular functioning, enzymes facilitating the formation of cholesterol intermediates could be specifically knocked out *in vitro* with pharmacological inhibitors, as was demonstrated by Dunn and colleagues(Dunn et al., 2006). Subsequently, reactions of T cells could be observed or measured and compared with T cells in which enzymes were not inhibited.

SREBP isoforms

Also unknown is the contribution of the different SREBP isoforms to the activity of the cholesterol biosynthesis pathway. While SREBP-1c is involved in fatty acid synthesis, SREBP-2 transcribes genes involved in cholesterol synthesis. The SREBP-1a isoform controls both the fatty acid and cholesterol pathway(Eberlé et al., 2004). While research on cholesterol in T cells has mainly focussed on SREBP-2, it is interesting to investigate SREBP-1a to answer the question: What is the relative contribution of SREBP-1a and SREBP2 on the amount of cholesterol biosynthesis in T cells?

This question could be answered by knocking out the SREBP-1a or the SREBP2 gene in human T cells *in vitro* and measuring the amount of HMGCR mRNA and protein. The HMGCR mRNA and protein quantity provides information about the activity of the cholesterol biosynthesis pathway.

Answering this question would clarify the relationship between cholesterol biosynthesis and fatty acid synthesis in different types of T cells. For example, Th17 cells are known to display both in increased cholesterol and fatty acid biosynthesis, while Th1 cells do not need fatty acid synthesis for their functioning(Geltink et al., 2018).

Effects of statins on T cells

Hypercholesterolemic and Tangier disease patients are often treated with statins. Statins inhibit HMGCR and thus lower cholesterol biosynthesis. Additionally, statins also increase the expression of LDLR in liver cells of patients without a homozygous defect for LDLR(Sirtori, 2014). There are no indications that the LDLR expression on T cells is also upregulated by statins(Raungaard et al., 2000; Tada et al., 2009). Nevertheless, an increased LDLR expression in hepatocytes can change cholesterol homeostasis in the whole body, thus influencing T cells.

Based on the HMGCR-inhibiting effect of statins, statins hypothetically decrease T cell activation and proliferation (section 3 and 4) and suppress differentiation of Th1, Th17, Treg, and CTL cells (section 5). However, studies on the effects of statins on T cells are contrasting. While in one study, statins reduce T cell activation and shift the Th1/Th2 balance towards Th2 in hypercholesterolemia in human peripheral blood mononuclear cells(Emruzi et al., 2019), other studies indicate a statin-mediated Th2 suppression in humans *in vivo*(Cherfan et al., 2007) and *in vitro*(Inagaki-Katashiba et al., 2019). As could be expected from Th2 suppression, statin use has been associated with decreased exacerbations in allergic asthma(Huang et al., 2011; J.-Y. Wang et al., 2018). Concerning Tregs, statins seem to increase their number in humans, but not in mice(Mausner-Fainberg et al., 2008).

An important outstanding question to be answered is therefore: how do statins alter T cell function in humans? Answering this question will increase the understanding of the mechanism of statins, which may aid in determining which patients will benefit most from statin treatment. Statin-mediated effects on T cells may have beneficial effects on atherogenesis development in patients with hypercholesterolemia.

A related outstanding question is: how do statins alter T cell receptor expression in homozygous FH patients? Since patients with a homozygous defect in the LDLR gene do not express functional LDLRs, statins will not increase the expression of functional LDLRs either. Possibly, alternative receptors are involved in lipoprotein uptake. An increased expression of alternative receptors upon statin treatment in homozygous FH patients would explain the LDL-cholesterol lowering effects of statins in these patients(Adhyaru & Jacobson, 2018).

Possible alternative receptors are scavenger receptor B1 (SR-B1), which plays a major role in HDL uptake by hepatocytes, but also recognizes LDL particles. Another potential alternative receptor in T cells is the cluster of differentiation 36 (CD36) receptor. CD36 is known for the uptake of oxidized LDL particles, but is also able to mediate the uptake of unoxidized LDL(Rhainds & Brissette, 1999). T cells are known to express SR-B1 and CD36(Barth et al., 2008; H. Wang et al., 2020). It remains to be understood whether SR-B1 and CD36 are upregulated in T cells in FH patients and how statins influence SR-B1 and CD36 expression.

Translation from mice to humans

Much research until now concerning cholesterol metabolism in T cells is done in mice models. Ldlr-/- mice and Apoe-/- mice are frequently used as a model for hypercholesterolemia. This way, T cell functioning in WT mice can be compared with hypercholesterolemic mice. Unfortunately, the lipid

profile in mice does not fully represent that in humans. While in humans LDL is the most abundant lipoprotein, mice carry cholesterol mostly by HDL. Moreover, while humans synthesize cholesterol mostly in extrahepatic tissues, cholesterol synthesis in mice is done primarily in the liver(Y. Zhao et al., 2020). These differences make it hard to translate results obtained in mice directly to humans. The question to answer is: do the known effects of cholesterol on T cells in mice apply for humans?

Alternative animal models to study cholesterol metabolism are the hamster and Guinee pig(Y. Zhao et al., 2020) or humanized mice models(Ellis et al., 2013). Research in humans themselves would be even better. Peripheral T lymphocytes could be easily isolated from human blood and used for further analysis(Kizhakeyil et al., 2019). For example, T cells from patients with defective cholesterol metabolism, including hypercholesterolemia, Tangier disease, and SLOS, can be compared with T cells from healthy humans.

7.3 Concluding remark

The current review has provided an overview of the influences of cholesterol on T cells, both in health and disease. Much research has proved that cholesterol plays a major role in T cells and that this has consequences for diseases in which cholesterol metabolism is disrupted. However, the whole picture is not yet complete and additional research is needed. A full understanding of cholesterol metabolism in T cells will aid in the development of efficient therapies, both in the highly prevalent disease hypercholesterolemia and in rare diseases like Tangier and SLOS.

Literature

Adhyaru, B. B., & Jacobson, T. A. (2018). Safety and efficacy of statin therapy. *Nature Reviews. Cardiology*, *15*(12), 757–769. https://doi.org/10.1038/s41569-018-0098-5

Aguilar-Ballester, M., Herrero-Cervera, A., Vinué, Á., Martínez-Hervás, S., & González-Navarro, H. (2020). Impact of Cholesterol Metabolism in Immune Cell Function and Atherosclerosis. *Nutrients*, *12*(7), E2021. https://doi.org/10.3390/nu12072021

Armstrong, A. J., Gebre, A. K., Parks, J. S., & Hedrick, C. C. (2010). ATP-binding cassette transporter G1 negatively regulates thymocyte and peripheral lymphocyte proliferation. *Journal of Immunology (Baltimore, Md.: 1950)*, *184*(1), 173–183. https://doi.org/10.4049/jimmunol.0902372

Balajthy, A., Somodi, S., Pethő, Z., Péter, M., Varga, Z., Szabó, G. P., Paragh, G., Vígh, L., Panyi, G., & Hajdu, P. (2016). 7DHC-induced changes of Kv1.3 operation contributes to modified T cell function in Smith-Lemli-Opitz syndrome. *Pflügers Archiv - European Journal of Physiology*, *468*(8), 1403–1418. https://doi.org/10.1007/s00424-016-1851-4

Barth, H., Schnober, E. K., Neumann-Haefelin, C., Thumann, C., Zeisel, M. B., Diepolder, H. M., Hu, Z., Liang, T. J., Blum, H. E., Thimme, R., Lambotin, M., & Baumert, T. F. (2008). Scavenger receptor class B is required for hepatitis C virus uptake and cross-presentation by human dendritic cells. *Journal of Virology*, *82*(7), 3466–3479. https://doi.org/10.1128/JVI.02478-07

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., Chamberlain, A. M., Chang, A. R., Cheng, S., Das, S. R., Delling, F. N., Djousse, L., Elkind, M. S. V., Ferguson, J. F., Fornage, M., Jordan, L. C., Khan, S. S., Kissela, B. M., Knutson, K. L., … American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. (2019). Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation*, *139*(10), e56–e528. https://doi.org/10.1161/CIR.0000000000000659

Bensinger, S. J., Bradley, M. N., Joseph, S. B., Zelcer, N., Janssen, E. M., Hausner, M. A., Shih, R., Parks, J. S., Edwards, P. A., Jamieson, B. D., & Tontonoz, P. (2008). LXR Signaling Couples Sterol Metabolism to Proliferation in the Acquired Immune Response. *Cell*, *134*(1), 97–111. https://doi.org/10.1016/j.cell.2008.04.052

Bi, X., Vitali, C., & Cuchel, M. (2015). ABCA1 and Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(7), 1551–1553. https://doi.org/10.1161/ATVBAHA.115.305547

Bietz, A., Zhu, H., Xue, M., & Xu, C. (2017). Cholesterol Metabolism in T Cells. *Frontiers in Immunology*, *8*, 1664. https://doi.org/10.3389/fimmu.2017.01664

Bochem, A. E., van der Valk, F. M., Tolani, S., Stroes, E. S., Westerterp, M., & Tall, A. R. (2015). Increased Systemic and Plaque Inflammation in ABCA1 Mutation Carriers With Attenuation by Statins. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(7), 1663–1669. https://doi.org/10.1161/ATVBAHA.114.304959

Bouhairie, V. E., & Goldberg, A. C. (2015). Familial Hypercholesterolemia. *Cardiology Clinics*, *33*(2), 169–179. https://doi.org/10.1016/j.ccl.2015.01.001

Bovenga, F., Sabbà, C., & Moschetta, A. (2015). Uncoupling Nuclear Receptor LXR and Cholesterol Metabolism in Cancer. *Cell Metabolism*, *21*(4), 517–526. https://doi.org/10.1016/j.cmet.2015.03.002

Brown, M. S., Radhakrishnan, A., & Goldstein, J. L. (2018). Retrospective on Cholesterol Homeostasis: The Central Role of Scap. *Annual Review of Biochemistry*, *87*, 783–807. https://doi.org/10.1146/annurev-biochem-062917-011852

Brumeanu, T.-D., Preda-Pais, A., Stoica, C., Bona, C., & Casares, S. (2007). Differential partitioning and trafficking of GM gangliosides and cholesterol-rich lipid rafts in thymic and splenic CD4 T cells. *Molecular Immunology*, *44*(4), 530–540. https://doi.org/10.1016/j.molimm.2006.02.008

Cai, Z., Ishibashi, T., Kozai, M., Mita, H., Wang, S., Takada, K., & Inaba, M. (2021). ROR agonist hampers the proliferation and survival of postactivated CD8+ T cells through the downregulation of cholesterol synthesis-related genes. *Immunology and Cell Biology*, *99*(3), 288–298. https://doi.org/10.1111/imcb.12406

Chan, D. C., & Watts, G. F. (2006). Recent advances in the investigation of lipoprotein metabolism using tracer methodology. *Clinical Laboratory*, *52*(7–8), 353–361.

Chang, J. T., Palanivel, V. R., Kinjyo, I., Schambach, F., Intlekofer, A. M., Banerjee, A., Longworth, S. A., Vinup, K. E., Mrass, P., Oliaro, J., Killeen, N., Orange, J. S., Russell, S. M., Weninger, W., & Reiner, S. L. (2007). Asymmetric T Lymphocyte Division in the Initiation of Adaptive Immune Responses. *Science*, *315*(5819), 1687–1691. https://doi.org/10.1126/science.1139393

Cheng, H.-Y., Gaddis, D. E., Wu, R., McSkimming, C., Haynes, L. D., Taylor, A. M., McNamara, C. A., Sorci-Thomas, M., & Hedrick, C. C. (2016). Loss of ABCG1 influences regulatory T cell differentiation and atherosclerosis. *The Journal of Clinical Investigation*, *126*(9), 3236–3246. https://doi.org/10.1172/JCI83136

Cheng, H.-Y., Wu, R., Gebre, A. K., Hanna, R. N., Smith, D. J., Parks, J. S., Ley, K., & Hedrick, C. C. (2013). Increased cholesterol content in gammadelta (γδ) T lymphocytes differentially regulates their activation. *PloS One*, *8*(5), e63746. https://doi.org/10.1371/journal.pone.0063746

Cherfan, P., Tompa, A., Wikby, A., Löfgren, S., & Jonasson, L. (2007). Effects of simvastatin on human T cells in vivo. *Atherosclerosis*, *193*(1), 186–192. https://doi.org/10.1016/j.atherosclerosis.2006.06.022

Crispe, I. N. (2009). The liver as a lymphoid organ. *Annual Review of Immunology*, *27*, 147–163. https://doi.org/10.1146/annurev.immunol.021908.132629

Cui, G., Qin, X., Wu, L., Zhang, Y., Sheng, X., Yu, Q., Sheng, H., Xi, B., Zhang, J. Z., & Zang, Y. Q. (2011). Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. *The Journal of Clinical Investigation*, *121*(2), 658–670. https://doi.org/10.1172/JCI42974

DeBarber, A. E., Eroglu, Y., Merkens, L. S., Pappu, A. S., & Steiner, R. D. (2011). Smith-Lemli-Opitz Syndrome. *Expert Reviews in Molecular Medicine*, *13*, e24. https://doi.org/10.1017/S146239941100189X

Delgoffe, G. M., Kole, T. P., Zheng, Y., Zarek, P. E., Matthews, K. L., Xiao, B., Worley, P. F., Kozma, S. C., & Powell, J. D. (2009). The mTOR Kinase Differentially Regulates Effector and Regulatory T Cell Lineage Commitment. *Immunity*, *30*(6), 832–844. https://doi.org/10.1016/j.immuni.2009.04.014

Dunn, S. E., Youssef, S., Goldstein, M. J., Prod'homme, T., Weber, M. S., Zamvil, S. S., & Steinman, L. (2006). Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. *Journal of Experimental Medicine*, *203*(2), 401–412. https://doi.org/10.1084/jem.20051129

Eberlé, D., Hegarty, B., Bossard, P., Ferré, P., & Foufelle, F. (2004). SREBP transcription factors: Master regulators of lipid homeostasis. *Biochimie*, *86*(11), 839–848. https://doi.org/10.1016/j.biochi.2004.09.018

Ellis, E. C. S., Nauglers, S., Parini, P., Mörk, L.-M., Jorns, C., Zemack, H., Sandblom, A. L., Björkhem, I., Ericzon, B.-G., Wilson, E. M., Strom, S. C., & Grompe, M. (2013). Mice with Chimeric Livers Are an Improved Model for Human Lipoprotein Metabolism. *PLOS ONE*, *8*(11), e78550. https://doi.org/10.1371/journal.pone.0078550

Emruzi, Z., Babaheidarian, P., Arshad, M., Stockinger, H., & Ahangari, G. (2019). Immune Modulatory Effects of Hypercholesterolemia: Can Atorvastatin Convert the Detrimental Effect of Hypercholesterolemia on the Immune System? *Iranian Journal of Allergy, Asthma, and Immunology*, *18*(5), 554–566. https://doi.org/10.18502/ijaai.v18i5.1925

Fitzky, B. U., Moebius, F. F., Asaoka, H., Waage-Baudet, H., Xu, L., Xu, G., Maeda, N., Kluckman, K., Hiller, S., Yu, H., Batta, A. K., Shefer, S., Chen, T., Salen, G., Sulik, K., Simoni, R. D., Ness, G. C., Glossmann, H., Patel, S. B., & Tint, G. S. (2001). 7-Dehydrocholesterol–dependent proteolysis

of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. *Journal of Clinical Investigation*, *108*(6), 905–915.

Gaddis, D. E., Padgett, L. E., Wu, R., McSkimming, C., Romines, V., Taylor, A. M., McNamara, C. A., Kronenberg, M., Crotty, S., Thomas, M. J., Sorci-Thomas, M. G., & Hedrick, C. C. (2018). Apolipoprotein AI prevents regulatory to follicular helper T cell switching during atherosclerosis. *Nature Communications*, *9*(1), 1095. https://doi.org/10.1038/s41467-018-03493-5

Gamba, P., Testa, G., Sottero, B., Gargiulo, S., Poli, G., & Leonarduzzi, G. (2012). The link between altered cholesterol metabolism and Alzheimer's disease. *Annals of the New York Academy of Sciences*, *1259*, 54–64. https://doi.org/10.1111/j.1749-6632.2012.06513.x

Geltink, R. I. K., Kyle, R. L., & Pearce, E. L. (2018). Unraveling the Complex Interplay Between T Cell Metabolism and Function. *Annual Review of Immunology*, *36*, 461–488. https://doi.org/10.1146/annurev-immunol-042617-053019

Goldstein, J. L., & Brown, M. S. (1973). Familial hypercholesterolemia: Identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. *Proceedings of the National Academy of Sciences of the United States of America*, *70*(10), 2804–2808. https://doi.org/10.1073/pnas.70.10.2804

Grünler, J., Ericsson, J., & Dallner, G. (1994). Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *1212*(3), 259–277. https://doi.org/10.1016/0005-2760(94)90200-3

Hooper, A. J., Hegele, R. A., & Burnett, J. R. (2020). Tangier disease: Update for 2020. *Current Opinion in Lipidology*, *31*(2), 80–84. https://doi.org/10.1097/MOL.0000000000000669

Hsieh, C. S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A., & Murphy, K. M. (1993). Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science (New York, N.Y.)*, *260*(5107), 547–549. https://doi.org/10.1126/science.8097338

Hu, X., Wang, Y., Hao, L.-Y., Liu, X., Lesch, C. A., Sanchez, B. M., Wendling, J. M., Morgan, R. W., Aicher, T. D., Carter, L. L., Toogood, P. L., & Glick, G. D. (2015). Sterol metabolism controls T(H)17 differentiation by generating endogenous RORγ agonists. *Nature Chemical Biology*, *11*(2), 141–147. https://doi.org/10.1038/nchembio.1714

Huang, C.-C., Chan, W.-L., Chen, Y.-C., Chen, T.-J., Chou, K.-T., Lin, S.-J., Chen, J.-W., & Leu, H.-B. (2011). Statin use in patients with asthma – a nationwide population-based study. *European Journal of Clinical Investigation*, *41*(5), 507–512. https://doi.org/10.1111/j.1365-2362.2010.02434.x

Hussain, M. M. (2014). Intestinal lipid absorption and lipoprotein formation. *Current Opinion in Lipidology*, *25*(3), 200–206. https://doi.org/10.1097/MOL.0000000000000084

Inagaki-Katashiba, N., Ito, T., Inaba, M., Azuma, Y., Tanaka, A., Phan, V., Kibata, K., Satake, A., & Nomura, S. (2019). Statins can suppress DC-mediated Th2 responses through the repression of OX40-ligand and CCL17 expression. *European Journal of Immunology*, *49*(11), 2051–2062. https://doi.org/10.1002/eji.201847992

Inoue, J., Sato, R., & Maeda, M. (1998). Multiple DNA elements for sterol regulatory element-binding protein and NF-Y are responsible for sterol-regulated transcription of the genes for human 3-hydroxy-3-methylglutaryl coenzyme A synthase and squalene synthase. *Journal of Biochemistry*, *123*(6), 1191–1198. https://doi.org/10.1093/oxfordjournals.jbchem.a022060

Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). Generation of lymphocytes in bone marrow and thymus. *Immunobiology: The Immune System in Health and Disease. 5th Edition*. https://www.ncbi.nlm.nih.gov/books/NBK27123/

Kelley, R. I., & Hennekam, R. C. M. (2000). The Smith-Lemli-Opitz syndrome. *Journal of Medical Genetics*, *37*(5), 321–335. https://doi.org/10.1136/jmg.37.5.321

Kidani, Y., Elsaesser, H., Hock, M. B., Vergnes, L., Williams, K. J., Argus, J. P., Marbois, B. N., Komisopoulou, E., Wilson, E. B., Osborne, T. F., Graeber, T. G., Reue, K., Brooks, D. G., & Bensinger, S. J. (2013). The sterol regulatory element binding proteins are essential for the

metabolic programming of effector T cells and adaptive immunity. *Nature Immunology*, *14*(5), 489–499. https://doi.org/10.1038/ni.2570

Kizhakeyil, A., Ong, S. T., Fazil, M. H. U. T., Chalasani, M. L. S., Prasannan, P., & Verma, N. K. (2019). Isolation of Human Peripheral Blood T-Lymphocytes. *Methods in Molecular Biology (Clifton, N.J.)*, *1930*, 11–17. https://doi.org/10.1007/978-1-4939-9036-8_2

Kovarova, M., Wassif, C. A., Odom, S., Liao, K., Porter, F. D., & Rivera, J. (2006). Cholesterol deficiency in a mouse model of Smith-Lemli-Opitz syndrome reveals increased mast cell responsiveness. *The Journal of Experimental Medicine*, *203*(5), 1161–1171. https://doi.org/10.1084/jem.20051701

Lee, H. J., Li, J., Vickman, R. E., Li, J., Liu, R., Durkes, A. C., Elzey, B. D., Yue, S., Liu, X., Ratliff, T. L., & Cheng, J.-X. (2018). Cholesterol Esterification Inhibition Suppresses Prostate Cancer Metastasis by Impairing the Wnt/β-catenin Pathway. *Molecular Cancer Research: MCR*, *16*(6), 974–985. https://doi.org/10.1158/1541-7786.MCR-17-0665

Levinson, S. S. (2020). Non–High-Density Lipoprotein Cholesterol and Guidelines for Cholesterol Lowering in Recent History. *Laboratory Medicine*, *51*(1), 14–23. https://doi.org/10.1093/labmed/lmz032

Li, M., Yang, Y., Wei, J., Cun, X., Lu, Z., Qiu, Y., Zhang, Z., & He, Q. (2018). Enhanced chemo-immunotherapy against melanoma by inhibition of cholesterol esterification in CD8+ T cells. *Nanomedicine: Nanotechnology, Biology and Medicine*, *14*(8), 2541–2550. https://doi.org/10.1016/j.nano.2018.08.008

Lim, H., Kim, Y. U., Sun, H., Lee, J. H., Reynolds, J. M., Hanabuchi, S., Wu, H., Teng, B.-B., & Chung, Y. (2014). Proatherogenic Conditions Promote Autoimmune T Helper 17 Cell Responses In Vivo. *Immunity*, *40*(1), 153–165. https://doi.org/10.1016/j.immuni.2013.11.021

Liu, W., Li, W.-M., Gao, C., & Sun, N.-L. (2005). Effects of atorvastatin on the Th1/Th2 polarization of ongoing experimental autoimmune myocarditis in Lewis rats. *Journal of Autoimmunity*, *25*(4), 258–263. https://doi.org/10.1016/j.jaut.2005.06.005

Lo Sasso, G., Schlage, W. K., Boué, S., Veljkovic, E., Peitsch, M. C., & Hoeng, J. (2016). The Apoe−/− mouse model: A suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. *Journal of Translational Medicine*, *14*, 146. https://doi.org/10.1186/s12967-016-0901-1

Lochner, M., Berod, L., & Sparwasser, T. (2015). Fatty acid metabolism in the regulation of T cell function. *Trends in Immunology*, *36*(2), 81–91. https://doi.org/10.1016/j.it.2014.12.005

Ludewig, B., Jäggi, M., Dumrese, T., Brduscha-Riem, K., Odermatt, B., Hengartner, H., & Zinkernagel, R. M. (2001). Hypercholesterolemia Exacerbates Virus-Induced Immunopathologic Liver Disease Via Suppression of Antiviral Cytotoxic T Cell Responses. *The Journal of Immunology*, *166*(5), 3369–3376. https://doi.org/10.4049/jimmunol.166.5.3369

Luo, J., Yang, H., & Song, B.-L. (2020). Mechanisms and regulation of cholesterol homeostasis. *Nature Reviews. Molecular Cell Biology*, *21*(4), 225–245. https://doi.org/10.1038/s41580-019-0190-7

Ma, K. L., Liu, J., Wang, C. X., Ni, J., Zhang, Y., Wu, Y., Lv, L. L., Ruan, X. Z., & Liu, B. C. (2013). Activation of mTOR modulates SREBP-2 to induce foam cell formation through increased retinoblastoma protein phosphorylation. *Cardiovascular Research*, *100*(3), 450–460. https://doi.org/10.1093/cvr/cvt203

Ma, X., Bi, E., Lu, Y., Su, P., Huang, C., Liu, L., Wang, Q., Yang, M., Kalady, M. F., Qian, J., Zhang, A., Gupte, A. A., Hamilton, D. J., Zheng, C., & Yi, Q. (2019). Cholesterol Induces CD8+ T Cell Exhaustion in the Tumor Microenvironment. *Cell Metabolism*, *30*(1), 143-156.e5. https://doi.org/10.1016/j.cmet.2019.04.002

MacIver, N. J., Jacobs, S. R., Wieman, H. L., Wofford, J. A., Coloff, J. L., & Rathmell, J. C. (2008). Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *Journal of Leukocyte Biology*, *84*(4), 949–957. https://doi.org/10.1189/jlb.0108024

Madsen, C. M., Varbo, A., & Nordestgaard, B. G. (2019). Low HDL Cholesterol and High Risk of Autoimmune Disease: Two Population-Based Cohort Studies Including 117341 Individuals. *Clinical Chemistry*, *65*(5), 644–652. https://doi.org/10.1373/clinchem.2018.299636

Maganto-García, E., Tarrio, M. L., Grabie, N., Bu, D., & Lichtman, A. H. (2011). Dynamic Changes in Regulatory T Cells Are Linked to Levels of Diet-Induced Hypercholesterolemia. *Circulation*, *124*(2), 185–195. https://doi.org/10.1161/CIRCULATIONAHA.110.006411

Mahnke, Y. D., Brodie, T. M., Sallusto, F., Roederer, M., & Lugli, E. (2013). The who's who of T-cell differentiation: Human memory T-cell subsets. *European Journal of Immunology*, *43*(11), 2797–2809. https://doi.org/10.1002/eji.201343751

Mailer, R. K. W., Gisterå, A., Polyzos, K. A., Ketelhuth, D. F. J., & Hansson, G. K. (2017a). Hypercholesterolemia Induces Differentiation of Regulatory T Cells in the Liver. *Circulation Research*, *120*(11), 1740–1753. https://doi.org/10.1161/CIRCRESAHA.116.310054

Mailer, R. K. W., Gisterå, A., Polyzos, K. A., Ketelhuth, D. F. J., & Hansson, G. K. (2017b). Hypercholesterolemia Enhances T Cell Receptor Signaling and Increases the Regulatory T Cell Population. *Scientific Reports*, *7*(1), 15655. https://doi.org/10.1038/s41598-017-15546-8

Mannu, G., Zaman, M., Gupta, A., HU, R., & Myint, P. (2013). Evidence of Lifestyle Modification in the Management of Hypercholesterolemia. *Current Cardiology Reviews*, *9*(1), 2–14. https://doi.org/10.2174/157340313805076313

Manti, S., Cuppari, C., Marseglia, L., D'Angelo, G., Arrigo, T., Gitto, E., Leonardi, S., & Salpietro, C. (2017). Association between Allergies and Hypercholesterolemia: A Systematic Review. *International Archives of Allergy and Immunology*, *174*(2), 67–76. https://doi.org/10.1159/000480081

Mausner-Fainberg, K., Luboshits, G., Mor, A., Maysel-Auslender, S., Rubinstein, A., Keren, G., & George, J. (2008). The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells. *Atherosclerosis*, *197*(2), 829–839. https://doi.org/10.1016/j.atherosclerosis.2007.07.031

Molnár, E., Swamy, M., Holzer, M., Beck-García, K., Worch, R., Thiele, C., Guigas, G., Boye, K., Luescher, I. F., Schwille, P., Schubert, R., & Schamel, W. W. A. (2012). Cholesterol and Sphingomyelin Drive Ligand-independent T-cell Antigen Receptor Nanoclustering. *Journal of Biological Chemistry*, *287*(51), 42664–42674. https://doi.org/10.1074/jbc.M112.386045

Ness, G. C., Lopez, D., Borrego, O., & Gilbert-Barness, E. (1997). Increased expression of low-density lipoprotein receptors in a Smith-Lemli-Opitz infant with elevated bilirubin levels. *American Journal of Medical Genetics*, *68*(3), 294–299. https://doi.org/10.1002/(sici)1096-8628(19970131)68:3<294::aid-ajmg9>3.0.co;2-m

Nguyen, L. B., Salen, G., Shefer, S., Tint, S. S., Shore, V., & Ness, G. C. (1990). Decreased cholesterol biosynthesis in sitosterolemia with xanthomatosis: Diminished mononuclear leukocyte 3-hydroxy-3-methylglutaryl coenzyme a reductase activity and enzyme protein associated with increased low-density lipoprotein receptor function. *Metabolism*, *39*(4), 436–443. https://doi.org/10.1016/0026-0495(90)90260-J

Ouimet, M., Barrett, T. J., & Fisher, E. A. (2019). HDL and Reverse Cholesterol Transport. *Circulation Research*, *124*(10), 1505–1518. https://doi.org/10.1161/CIRCRESAHA.119.312617

Pageon, S. V., Tabarin, T., Yamamoto, Y., Ma, Y., Nicovich, P. R., Bridgeman, J. S., Cohnen, A., Benzing, C., Gao, Y., Crowther, M. D., Tungatt, K., Dolton, G., Sewell, A. K., Price, D. A., Acuto, O., Parton, R. G., Gooding, J. J., Rossy, J., Rossjohn, J., & Gaus, K. (2016). Functional role of T-cell receptor nanoclusters in signal initiation and antigen discrimination. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(37), E5454–E5463. https://doi.org/10.1073/pnas.1607436113

Parigi, S. M., Das, S., Frede, A., Cardoso, R. F., Tripathi, K. P., Doñas, C., Hu, Y. O. O., Antonson, P., Engstrand, L., Gustafsson, J.-Å., & Villablanca, E. J. (2021). Liver X receptor regulates Th17 and RORγt+ Treg cells by distinct mechanisms. *Mucosal Immunology*, *14*(2), 411–419. https://doi.org/10.1038/s41385-020-0323-5

Park, J.-S., Moon, S.-J., Lim, M.-A., Byun, J.-K., Hwang, S.-H., Yang, S., Kim, E.-K., Lee, H., Kim, S.-M., Lee, J., Kwok, S.-K., Min, J.-K., Lee, M.-O., Shin, D.-Y., Park, S.-H., & Cho, M.-L. (2019). Retinoic Acid Receptor-Related Receptor Alpha Ameliorates Autoimmune Arthritis via Inhibiting of Th17 Cells and Osteoclastogenesis. *Frontiers in Immunology*, *10*, 2270. https://doi.org/10.3389/fimmu.2019.02270

Pendse, A. A., Arbones-Mainar, J. M., Johnson, L. A., Altenburg, M. K., & Maeda, N. (2009). Apolipoprotein E knock-out and knock-in mice: Atherosclerosis, metabolic syndrome, and beyond. *Journal of Lipid Research*, *50*(Suppl), S178–S182. https://doi.org/10.1194/jlr.R800070-JLR200

Perucha, E., Melchiotti, R., Bibby, J. A., Wu, W., Frederiksen, K. S., Roberts, C. A., Hall, Z., LeFriec, G., Robertson, K. A., Lavender, P., Gerwien, J. G., Taams, L. S., Griffin, J. L., de Rinaldis, E., van Baarsen, L. G. M., Kemper, C., Ghazal, P., & Cope, A. P. (2019). The cholesterol biosynthesis pathway regulates IL-10 expression in human Th1 cells. *Nature Communications*, *10*(1), 498. https://doi.org/10.1038/s41467-019-08332-9

Pinkwart, K., Schneider, F., Lukoseviciute, M., Sauka-Spengler, T., Lyman, E., Eggeling, C., & Sezgin, E. (2019). Nanoscale dynamics of cholesterol in the cell membrane. *The Journal of Biological Chemistry*, *294*(34), 12599–12609. https://doi.org/10.1074/jbc.RA119.009683

Pollizzi, K. N., Sun, I.-H., Patel, C. H., Lo, Y.-C., Oh, M.-H., Waickman, A. T., Tam, A. J., Blosser, R. L., Wen, J., Delgoffe, G. M., & Powell, J. D. (2016). Asymmetric inheritance of mTORC1 kinase activity during division dictates CD8(+) T cell differentiation. *Nature Immunology*, *17*(6), 704–711. https://doi.org/10.1038/ni.3438

Raungaard, B., Brorholt-Petersen, J. U., Jensen, H. K., & Færgeman, O. (2000). Flow Cytometric Assessment of Effects of Fluvastatin on Low-Density Lipoprotein Receptor Activity in Stimulated T-Lymphocytes from Patients with Heterozygous Familial Hypercholesterolemia. *The Journal of Clinical Pharmacology*, *40*(4), 421–429. https://doi.org/10.1177/00912700022009008

Ray, J. P., Staron, M. M., Shyer, J. A., Ho, P.-C., Marshall, H. D., Gray, S. M., Laidlaw, B. J., Araki, K., Ahmed, R., Kaech, S. M., & Craft, J. (2015). The Interleukin-2-mTORc1 Kinase Axis Defines the Signaling, Differentiation, and Metabolism of T Helper 1 and Follicular B Helper T Cells. *Immunity*, *43*(4), 690–702. https://doi.org/10.1016/j.immuni.2015.08.017

Rhainds, D., & Brissette, L. (1999). Low density lipoprotein uptake: Holoparticle and cholesteryl ester selective uptake. *The International Journal of Biochemistry & Cell Biology*, *31*(9), 915–931. https://doi.org/10.1016/S1357-2725(99)00046-1

Rhoads, J. P., & Major, A. S. (2018). How Oxidized Low-Density Lipoprotein Activates Inflammatory Responses. *Critical Reviews in Immunology*, *38*(4), 333–342. https://doi.org/10.1615/CritRevImmunol.2018026483

Robertson, A.-K. L., Zhou, X., Strandvik, B., & Hansson, G. K. (2004). Severe Hypercholesterolaemia Leads to Strong Th2 Responses to an Exogenous Antigen. *Scandinavian Journal of Immunology*, *59*(3), 285–293. https://doi.org/10.1111/j.0300-9475.2004.01403.x

Ryu, H., Kim, J., Kim, D., Lee, J.-E., & Chung, Y. (2019). Cellular and Molecular Links between Autoimmunity and Lipid Metabolism. *Molecules and Cells*, *42*(11), 747–754. https://doi.org/10.14348/molcells.2019.0196

Saigusa, R., Winkels, H., & Ley, K. (2020). T cell subsets and functions in atherosclerosis. *Nature Reviews. Cardiology*, *17*(7), 387–401. https://doi.org/10.1038/s41569-020-0352-5

Santos, R. D. (2019). Screening and management of familial hypercholesterolemia. *Current Opinion in Cardiology*, *34*(5), 526–530. https://doi.org/10.1097/HCO.0000000000000660

Schade, D. S., Shey, L., & Eaton, R. P. (2020). Cholesterol Review: A Metabolically Important Molecule. *Endocrine Practice*, *26*(12), 1514–1523. https://doi.org/10.4158/EP-2020-0347

Seder, R. A., & Ahmed, R. (2003). Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. *Nature Immunology*, *4*(9), 835–842. https://doi.org/10.1038/ni969

Singh, P., Saxena, R., Srinivas, G., Pande, G., & Chattopadhyay, A. (2013). Cholesterol Biosynthesis and Homeostasis in Regulation of the Cell Cycle. *PLoS ONE*, *8*(3), e58833. https://doi.org/10.1371/journal.pone.0058833

Sirtori, C. R. (2014). The pharmacology of statins. *Pharmacological Research*, *88*, 3–11. https://doi.org/10.1016/j.phrs.2014.03.002

Smet, M., Van Hoecke, L., De Beuckelaer, A., Vander Beken, S., Naessens, T., Vergote, K., Willart, M., Lambrecht, B. N., Gustafsson, J., Steffensen, K. R., & Grooten, J. (2016). Cholesterol-sensing liver X receptors stimulate Th2-driven allergic eosinophilic asthma in mice. *Immunity, Inflammation and Disease*, *4*(3), 350–361. https://doi.org/10.1002/iid3.118

Suárez-Rivero, J. M., de la Mata, M., Pavón, A. D., Villanueva-Paz, M., Povea-Cabello, S., Cotán, D., Álvarez-Córdoba, M., Villalón-García, I., Ybot-González, P., Salas, J. J., Muñiz, O., Cordero, M. D., & Sánchez-Alcázar, J. A. (2018). Intracellular cholesterol accumulation and coenzyme Q10 deficiency in Familial Hypercholesterolemia. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1864*(12), 3697–3713. https://doi.org/10.1016/j.bbadis.2018.10.009

Surls, J., Nazarov-Stoica, C., Kehl, M., Olsen, C., Casares, S., & Brumeanu, T.-D. (2012). Increased Membrane Cholesterol in Lymphocytes Diverts T-Cells toward an Inflammatory Response. *PLoS ONE*, *7*(6), e38733. https://doi.org/10.1371/journal.pone.0038733

Swamy, M., Beck-Garcia, K., Beck-Garcia, E., Hartl, F. A., Morath, A., Yousefi, O. S., Dopfer, E. P., Molnár, E., Schulze, A. K., Blanco, R., Borroto, A., Martín-Blanco, N., Alarcon, B., Höfer, T., Minguet, S., & Schamel, W. W. A. (2016). A Cholesterol-Based Allostery Model of T Cell Receptor Phosphorylation. *Immunity*, *44*(5), 1091–1101. https://doi.org/10.1016/j.immuni.2016.04.011

Szabo, P. A., Levitin, H. M., Miron, M., Snyder, M. E., Senda, T., Yuan, J., Cheng, Y. L., Bush, E. C., Dogra, P., Thapa, P., Farber, D. L., & Sims, P. A. (2019). Single-cell transcriptomics of human T cells reveals tissue and activation signatures in health and disease. *Nature Communications*, *10*(1), 4706. https://doi.org/10.1038/s41467-019-12464-3

Tada, H., Kawashiri, M., Noguchi, T., Mori, M., Tsuchida, M., Takata, M., Nohara, A., Inazu, A., Kobayashi, J., Yachie, A., Mabuchi, H., & Yamagishi, M. (2009). A novel method for determining functional LDL receptor activity in familial hypercholesterolemia: Application of the CD3/CD28 assay in lymphocytes. *Clinica Chimica Acta*, *400*(1), 42–47. https://doi.org/10.1016/j.cca.2008.10.010

Tang, R., & Xu, Z. (2020). Gene therapy: A double-edged sword with great powers. *Molecular and Cellular Biochemistry*, *474*(1–2), 73–81. https://doi.org/10.1007/s11010-020-03834-3

Timilshina, M., You, Z., Lacher, S. M., Acharya, S., Jiang, L., Kang, Y., Kim, J.-A., Chang, H. W., Kim, K.-J., Park, B., Song, J.-H., Ko, H.-J., Park, Y.-Y., Ma, M.-J., Nepal, M. R., Jeong, T. C., Chung, Y., Waisman, A., & Chang, J.-H. (2019). Activation of Mevalonate Pathway via LKB1 Is Essential for Stability of Treg Cells. *Cell Reports*, *27*(10), 2948-2961.e7. https://doi.org/10.1016/j.celrep.2019.05.020

Turbitt, W. J., Rosean, C. B., Weber, K. S., & Norian, L. A. (2020). Obesity and CD8 T cell metabolism: Implications for anti-tumor immunity and cancer immunotherapy outcomes. *Immunological Reviews*, *295*(1), 203–219. https://doi.org/10.1111/imr.12849

Virani, S. S., Alonso, A., Aparicio, H. J., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., Carson, A. P., Chamberlain, A. M., Cheng, S., Delling, F. N., Elkind, M. S. V., Evenson, K. R., Ferguson, J. F., Gupta, D. K., Khan, S. S., Kissela, B. M., Knutson, K. L., Lee, C. D., Lewis, T. T., … American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. (2021). Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. *Circulation*, *143*(8), e254–e743. https://doi.org/10.1161/CIR.0000000000000950

Wang, F., Beck-García, K., Zorzin, C., Schamel, W. W. A., & Davis, M. M. (2016). Inhibition of T cell receptor signaling by cholesterol sulfate, a naturally occurring derivative of membrane cholesterol. *Nature Immunology*, *17*(7), 844–850. https://doi.org/10.1038/ni.3462

Wang, H., Franco, F., Tsui, Y.-C., Xie, X., Trefny, M. P., Zappasodi, R., Mohmood, S. R., Fernández-García, J., Tsai, C.-H., Schulze, I., Picard, F., Meylan, E., Silverstein, R., Goldberg, I., Fendt, S.-M., Wolchok, J. D., Merghoub, T., Jandus, C., Zippelius, A., & Ho, P.-C. (2020). CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nature Immunology*, *21*(3), 298–308. https://doi.org/10.1038/s41590-019-0589-5

Wang, J.-Y., Yao, T.-C., Tsai, Y.-T., Wu, A. C., & Tsai, H.-J. (2018). Increased Dose and Duration of Statin Use Is Associated with Decreased Asthma-Related Emergency Department Visits and Hospitalizations. *The Journal of Allergy and Clinical Immunology. In Practice*, *6*(5), 1588-1595.e1. https://doi.org/10.1016/j.jaip.2017.12.017

Wang, P., Zhang, Q., Tan, L., Xu, Y., Xie, X., & Zhao, Y. (2020). The Regulatory Effects of mTOR Complexes in the Differentiation and Function of CD4+ T Cell Subsets. *Journal of Immunology Research*, *2020*, 3406032. https://doi.org/10.1155/2020/3406032

West, E., Osborne, C., & Bate, C. (2017). The cholesterol ester cycle regulates signalling complexes and synapse damage caused by amyloid-β. *Journal of Cell Science*, *130*(18), 3050–3059. https://doi.org/10.1242/jcs.205484

Xu, L., Huang, Q., Wang, H., Hao, Y., Bai, Q., Hu, J., Li, Y., Wang, P., Chen, X., He, R., Li, B., Yang, X., Zhao, T., Zhang, Y., Wang, Y., Ou, J., Liang, H., Wu, Y., Zhou, X., & Ye, L. (2017). The Kinase mTORC1 Promotes the Generation and Suppressive Function of Follicular Regulatory T Cells. *Immunity*, *47*(3), 538-551.e5. https://doi.org/10.1016/j.immuni.2017.08.011

Yang, W., Bai, Y., Xiong, Y., Zhang, J., Chen, S., Zheng, X., Meng, X., Li, L., Wang, J., Xu, C., Yan, C., Wang, L., Chang, C. C. Y., Chang, T.-Y., Zhang, T., Zhou, P., Song, B.-L., Liu, W., Sun, S., … Xu, C. (2016). Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. *Nature*, *531*(7596), 651–655. https://doi.org/10.1038/nature17412

Youssef, S., Stüve, O., Patarroyo, J. C., Ruiz, P. J., Radosevich, J. L., Hur, E. M., Bravo, M., Mitchell, D. J., Sobel, R. A., Steinman, L., & Zamvil, S. S. (2002). The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature*, *420*(6911), 78–84. https://doi.org/10.1038/nature01158

Zeng, H., Cohen, S., Guy, C., Shrestha, S., Neale, G., Brown, S. A., Cloer, C., Kishton, R. J., Gao, X., Youngblood, B., Do, M., Li, M. O., Locasale, J. W., Rathmell, J. C., & Chi, H. (2016). MTORC1 and mTORC2 Kinase Signaling and Glucose Metabolism Drive Follicular Helper T Cell Differentiation. *Immunity*, *45*(3), 540–554. https://doi.org/10.1016/j.immuni.2016.08.017

Zeng, H., Yang, K., Cloer, C., Neale, G., Vogel, P., & Chi, H. (2013). MTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature*, *499*(7459), 485–490. https://doi.org/10.1038/nature12297

Zhao, Q., Duck, L. W., Huang, F., Alexander, K. L., Maynard, C. L., Mannon, P. J., & Elson, C. O. (2020). CD4+ T cell activation and concomitant mTOR metabolic inhibition can ablate microbiota-specific memory cells and prevent colitis. *Science Immunology*, *5*(54), eabc6373. https://doi.org/10.1126/sciimmunol.abc6373

Zhao, Y., Qu, H., Wang, Y., Xiao, W., Zhang, Y., & Shi, D. (2020). Small rodent models of atherosclerosis. *Biomedicine & Pharmacotherapy*, *129*, 110426. https://doi.org/10.1016/j.biopha.2020.110426

Zhou, X., Paulsson, G., Stemme, S., & Hansson, G. K. (1998). Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. *Journal of Clinical Investigation*, *101*(8), 1717–1725.

Zhu, J. (2018). T Helper Cell Differentiation, Heterogeneity, and Plasticity. *Cold Spring Harbor Perspectives in Biology*, *10*(10), a030338. https://doi.org/10.1101/cshperspect.a030338