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T-cell Cholesterol Accumulation, Aging, and Atherosclerosis

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Abstract

Purpose of Review The majority of leukocytes in advanced human atherosclerotic plaques are T-cells. T-cell subsets exert pro- or anti-atherogenic effects largely via the cytokines they secrete. $T_{regulatory}$ cells (T_{regs}) are anti-inflammatory, but may lose these properties during atherosclerosis, proposed to be downstream of cholesterol accumulation. Aged T-cells also accumulate cholesterol. The effects of T-cell cholesterol accumulation on T-cell fate and atherosclerosis are not uniform.

Recent findings T-cell cholesterol accumulation enhances differentiation into pro-atherogenic cytotoxic T-cells and boosts their killing capacity, depending on the localization and extent of cholesterol accumulation. Excessive cholesterol accumulation induces T-cell exhaustion or T-cell apoptosis, the latter decreasing atherosclerosis but impairing T-cell functionality in terms of killing capacity and proliferation. This may explain the compromised T-cell functionality in aged T-cells and T-cells from CVD patients.

Summary The extent of T-cell cholesterol accumulation and its cellular localization determine T-cell fate and downstream effects on atherosclerosis and T-cell functionality.

Keywords T-cell · Atherosclerosis · ABC transporters · Cholesterol · CVD

Introduction

T-cells make up ~50–65% of all leukocytes in advanced human atherosclerotic lesions from carotid endarterectomies [1••, 2••]. After infiltration into atherosclerotic lesions, T-cells interact with macrophages and dendritic cells (DCs) [3••]. Upon recognition of their cognate antigen presented by DCs and dependent on the cytokine milieu, naïve T-cells differentiate into distinct subsets characterized by the expression of transcription factors (i.e., FoxP3 for T regulatory cells (T_{regs}); T_{bet} for T helper 1 (T_h1) cells; GATA3 for T_h2 cells; retinoic acid-related orphan receptor (ROR)γT for T_h17 cells; and B-cell lymphoma (Bcl)6 for $T_{follicular\ helper}$ (T_{fh}) cells) [3••]. More than 80% of T-cells in atherosclerotic plaques express CD44, indicating that they are antigen experienced [2••, 3••]. Among the antigens that DCs present to T-cells are apolipoprotein B100 (apoB100), low-density lipoprotein (LDL), and oxidized LDL [4–6]. While initial studies have suggested that antigen presentation in atherosclerotic plaques induces production of the pro-atherogenic T_h1 cytokines interferon γ (IFNγ) and tumor necrosis factor α (TNFα) [4, 7], later studies have shown an expansion of T_{regs} in response to antigens [6, 8, 9]. T_{regs} exert an anti-atherogenic role by secreting interleukin (IL)-10 and transforming growth factor β (TGF-β) [10, 11]. TGF-β induces smooth muscle cell (SMC) migration and collagen production by SMCs [12–15]. Recent single-cell RNA sequencing (sc-RNA-Seq) studies have revealed a high diversity of T-cells in human atherosclerotic plaques [1••, 2••]. The role of specific T-cell subsets in atherosclerosis has been reviewed previously [3••].

Even though individual T-cell subsets have pro- or anti-atherogenic effects, presumably via the cytokines they secrete, complete $CD4^+$ or $CD8^+$ T-cell ablation reduces atherosclerosis in mice [16–19]; however, in advanced atherosclerosis, $CD8^+$ T-cell ablation increases plaque stability [20•], highlighting the complex role of T-cells in atherogenesis.

Recent studies have revealed that during atherosclerosis and cardiovascular disease (CVD) in humans, T_{regs} acquire

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markers of T_h1, T_h17, and T_{fh} cells, or switch to a more memory-like phenotype, which may render them pro-atherogenic [6, 9, 21, 22, 23•, 24]. Studies in mouse models have proposed that T-cell cholesterol accumulation critically contributes to this effect [23•]. In addition, T-cells from CVD patients lose their ability to proliferate and, therefore, to respond adequately to antigens [25•], a critical function of T-cells. The decreased proliferation may be the result of T-cell apoptosis downstream of excessive T-cell cholesterol accumulation [26••]. Aged T-cells also show increased cholesterol accumulation [27, 28]. Here, we will review how pathways that regulate T-cell cholesterol accumulation determine T-cell fate, atherosclerosis, and T-cell aging.

T-cell Receptor Stimulation and Cholesterol Accumulation

T-cells express high levels of the cholesterol transporters ATP Binding Cassette A1 (ABCA1) and ABCG1 that mediate cholesterol efflux to apolipoprotein A-I (apoA-I) and high-density lipoprotein (HDL), respectively [29]. T-cells mainly accumulate cholesterol in their plasma membrane, which is key to T-cell receptor (TCR) signaling and proliferation in response to interaction with their cognate antigen. TCR stimulation by anti-CD3, which mimics T-cell stimulation by antigen-presenting cells via major histocompatibility complex (MHC)I/II, decreases expression of the cholesterol transporters *Abca1* and *Abcg1* (Fig. 1) [30••, 31••]. The decreased expression of ABC cholesterol transporters is mediated by suppression of Liver X receptor (LXR) signaling due to upregulation of the enzyme sulfotransferase family cytosolic 2B member 1 (SULT2B1) that transfers sulfate groups to oxysterols, which inactivates oxysterols in terms of their ability to bind the transcription factor LXR and to activate it [30••, 32]. TCR stimulation also increases the expression of 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), the LDL receptor (*Ldlr*), and acetyl coA acyl transferase 1 (*Acat1*), which promote cholesterol synthesis, uptake, and esterification, respectively (Fig. 1) [30••, 31••].

T-cell Membrane Cholesterol Accumulation Induces T-cell Proliferation

Several lines of evidence indicate that cholesterol accumulation is key to T-cell proliferation and, as such, key to the T-cell response upon interaction with an antigen. Suppression of cholesterol synthesis due to deficiency of sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) completely abolishes T-cell proliferation in response to anti-CD3 [33•]. Conversely, when cholesterol

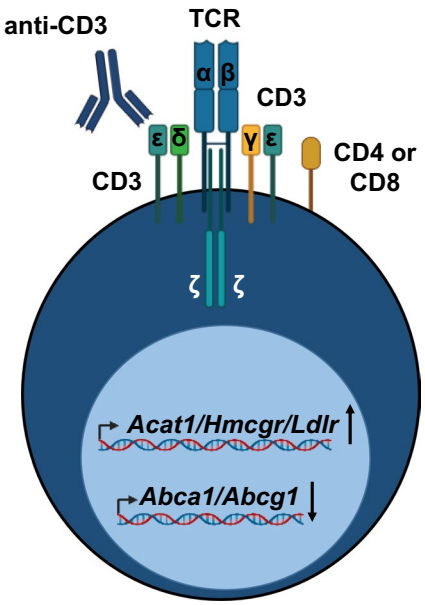

Fig. 1 Effects of anti-CD3 stimulation on expression of genes involved in cholesterol homeostasis. Gene transcription is shown in the nucleus. Created with BioRender.com

cannot be esterified due to deficiency of *Acat1*, plasma membrane cholesterol accumulation increases, as does T-cell proliferation [31••]. Similarly, deficiency of *Abcg1*-mediated cholesterol efflux promotes plasma membrane cholesterol accumulation and T-cell proliferation [30••, 34, 35]. T-cell cholesterol loading via methyl-β-cyclodextrin (MβCD)-cholesterol or LDL-cholesterol (LDL-c) also increases proliferation [34, 36].

Abcg1 deficient T-cells show high expression of *Abca1* [34], presumably due to the accumulation of oxysterols that induce the activation of LXR and consequently *Abca1* transcription [37,38]. Recent studies have revealed that T-cell *Abca1* deficiency increases *Abcg1* expression, reduces T-cell membrane cholesterol accumulation, and decreases T-cell proliferation in response to anti-CD3 [39]. These data suggest that, as initially proposed [30••], *Abcg1* is the dominant cholesterol transporter in T-cells. We found that deficiency of both *Abca1* and *Abcg1* increases T-cell membrane cholesterol accumulation and proliferation in young mice [26••]. Conversely, incubation with reconstituted HDL (rHDL) that induces cholesterol efflux, shows the opposite [26••]. Recent studies revealed that *histone deacetylase 3* (*Hdac3*) deficiency decreases T-cell proliferation, which was attributed to decreased membrane cholesterol accumulation and increased *Abca1* and *Abcg1* mRNA expression [40•]. These data substantiate the crucial role for cholesterol efflux pathways in regulating T-cell proliferation. An overview of pathways regulating cholesterol accumulation and T-cell proliferation is given in Table 1.

Table 1 Plasma membrane cholesterol and T-cell proliferation

Model	Plasma membrane cholesterol	T-cell proliferation	T-cell subtypes
Lxrβ deficiency [30••]	Not reported	↑	Total T-cells
Abcg1 deficiency [30••, 34, 35]	↑	↑	$CD4^+$
T-cell *Abca1* deficiency [39]	↓	↓	$CD4^+$ or $CD8^+$
T-cell *Acat1* deficiency [31••]	↑	↑	$CD8^+$
T-cell *Scap* deficiency [33•]	↓	↓	$CD8^+$
T-cell *Hdac3* deficiency [40•]	↓	↓	$CD4^+$
MβCD-cholesterol (20 µg/mL; 2 hours prior to TCR stimulus) [34]	Not reported	↑	$CD4^+$
LDL-c (72 hours during TCR stimulus) [36]	Not reported	↑	$CD8^+$
T-cell *Abca1/Abcg1* deficiency, young [26••]	↑	↑	$CD4^+$ or $CD8^+$
rHDL (50 µg/mL; 72 hours during TCR stimulus) [26••]	Not reported	↓	$CD4^+$ or $CD8^+$

T-cells were stimulated with αCD3/αCD28 for 72 h to induce proliferation; except for [34] (66 h)

Abca1 and Abcg1, ATP binding cassette A1 and G1; *Acat1*, acyl-CoA cholesterol acyltransferase 1; *Hdac3*, histone deacetylase 3; *LDL*, low-density lipoprotein; *Lxrβ*, liver X receptor β; *MβCD*-cholesterol, methyl-β-cyclodextrin cholesterol; *rHDL*, reconstituted high-density lipoprotein; *Scap*, sterol regulatory element-binding protein (SREBP) cleavage-activating protein; *TCR*, T-cell receptor

T-cell Proliferation During Aging and CVD

While combined T-cell *Abca1/Abcg1* deficiency increased T-cell proliferation in young mice, T-cell *Abca1/Abcg1* deficiency almost abolished T-cell proliferation in mice at 1 year of age, concomitant with an upregulation of the senescence marker p21 [26••]. These findings suggest that perhaps aged *Abca1/Abcg1* deficient T-cells became senescent due to several rounds of homeostatic proliferation. In addition, *Abca1/Abcg1* deficiency increased T-cell apoptosis, in both young mice and mice at 1 year of age [26••]. The increase in T-cell apoptosis may be more prominent during aging, as such contributing to the abolished T-cell proliferation in aged mice.

Interestingly, individuals over 70 years of age also show T-cell cholesterol accumulation compared to T-cells from individuals less than 25 years of age [27, 28], as do T-cells from wild-type mice at 2 years of age compared to T-cells from wild-type mice at 3 months of age [26••]. T-cells from aged mice (2 years) show increased apoptosis compared to T-cells from young mice (3 months) [26••]. Based on the findings in mice with T-cell *Abca1/Abcg1* deficiency [26••], these data suggest that also during aging, T-cell cholesterol accumulation contributes to apoptosis and, consequently, the decline in total T-cells. T-cell proliferation was only minimally decreased in T-cells from aged mice compared to young mice [26••]. However, T-cells from *Apolipoprotein e* deficient ($Apoe^{-/-}$) mice with advanced atherosclerosis due to 20 weeks of cholesterol-rich Western-type diet (WTD) feeding, show decreased T-cell proliferation and increased T-cell apoptosis compared to T-cells from $Apoe^{-/-}$ mice fed a chow diet [25•]. Even though this was attributed to impaired antigen presentation by DCs [25•], previous studies have shown that WTD feeding induces cholesterol accumulation in $Apoe^{-/-}$ T-cells [23•], and our studies in mice with T-cell *Abca1/Abcg1* deficiency demonstrate that T-cell cholesterol accumulation may directly increase T-cell apoptosis [26••].

In line with the findings in $Apoe^{-/-}$ mice, patients with advanced coronary artery disease (CAD) show a decrease in proliferation and an increase in T-cell apoptosis compared to patients with early CAD, irrespective of age (n = 14 patients per group) [25•]. While this would need to be confirmed in a larger CAD cohort, the data suggest a direct link between advanced CAD and impaired T-cell functionality due to T-cell apoptosis. Our data show that T-cell cholesterol accumulation, which may be aggravated in advanced CAD, contributes to this impaired T-cell functionality.

Not all genes that affect T-cell membrane cholesterol accumulation and TCR signaling (Table 1) affect apoptosis. *Acat1* deficiency decreased apoptosis in $CD8^+$ T-cells [31••], perhaps due to *Acat1* deficiency increasing T-cell proliferation and survival, which may offset potential effects on apoptosis. However, it should be noted that the effects of *Abca1/Abcg1* or *Apoe* were most pronounced in $CD4^+$ T-cells [25•, 26••], and are probably the consequence of an increase in intracellular T-cell membrane cholesterol accumulation that is more dramatic than reported for other genes listed in Table 1. Nonetheless, T-cell cholesterol accumulation induced by MβCD-cholesterol loading promotes endoplasmic reticulum (ER) stress and $CD8^+$ T-cell exhaustion without affecting apoptosis [41•]. We found that also in aged T-cells from wild-type mice (2 years old), expression of SREBP2 was decreased compared to T-cells from young mice (3 months), suggestive of ER cholesterol accumulation

[26••]. ER cholesterol accumulation may account for T-cell exhaustion during aging.

T-cell Membrane Cholesterol Accumulation and Differentiation into Cytotoxic T-cells

In addition to T-cell proliferation, TCR stimulation increases granzyme B, IFNγ, and TNFα positive $CD8^+$ T-cells, which are required for killing of foreign cells or pathogens [42]. Similar to effects on T-cell proliferation, deletion of genes or treatments that favor cholesterol accumulation (*Lxrβ* deficiency [30••], *Acat1* deficiency [31••], MβCD-cholesterol [31••], and LDL-c [36]) induce differentiation into these cytotoxic $CD8^+$ T-cells, while a decrease in cholesterol synthesis by *Scap* deficiency [33•] or treatment with lovastatin [31••] or cholesterol depletion by MβCD [31••] does the opposite. Also, inhibition of Niemann-Pick C1 protein, which induces movement of cholesterol from lysosomes to the plasma membrane, by the U18666A compound, decreases differentiation into these cytotoxic T-cells [31••], presumably due to decreased plasma membrane cholesterol [43]. These findings are summarized in Table 2. In line, T-cell *Abca1/Abcg1* deficiency induces differentiation into granzyme B and IFNγ expressing $CD8^+$ T-cells [26••]. However, T-cell *Abca1/Abcg1* deficiency decreased IFNγ secretion and T-cell mediated macrophage killing [26••]. We attributed these effects to increased T-cell apoptosis, and therefore these effects are simply the consequence of a lower number of T-cells [26••]. Similarly, T-cells from $Apoe^{-/-}$ mice fed WTD for 20 weeks show decreased IFNγ production compared to $Apoe^{-/-}$ mice fed a chow diet, concomitant with increased apoptosis [25•].

T-cell Membrane Cholesterol Accumulation, Atherosclerosis, and CVD

While effects of membrane cholesterol accumulation on T-cell proliferation and differentiation into cytotoxic T-cells seem to be relatively uniform, effects of T-cell cholesterol accumulation on downstream T-cell differentiation are not. T-cell *Abcg1* deficiency increases membrane cholesterol accumulation and lipid droplet formation, indicative of increased cholesterol esterification [35]. T-cell *Abcg1* deficiency increases formation of T_{regs}, with athero-protective effects [35].

Recent studies have revealed that during atherosclerosis and CVD, T_{regs} acquire markers of T_h1, T_h17, and T_{fh} cells, which may render them pro-atherogenic [6, 9, 21, 22, 23•, 24] (Fig. 2). Using a fluorescent tracing technique, current T_{regs} and exT_{regs} (cells that were T_{regs} before) could be distinguished in $Apoe^{-/-}$ mice [23•]. This revealed that upon WTD feeding, T_{regs} underwent a phenotypic switch [23•]. Injections of apoA-I reversed this switch [23•], and therefore this switch was proposed to occur downstream of cholesterol efflux and thus to be cholesterol-dependent. In this model, T_{regs} lost their Foxp3 and CD25 expression and started to express IFNγ or Bcl6 and IL-21, suggesting differentiation into T_h1 or T_{fh} cells, respectively [23•]. Previous sc-RNA-Seq studies have indeed shown that T_{regs} gain features of T_h1 cells during atherosclerosis in $Apoe^{-/-}$ mice and that these cells are dysfunctional in terms of suppressing T-cell proliferation, a main characteristic of T_{regs} [22]. Deficiency of the specific T_{fh} transcription factor *Bcl6* decreased atherosclerosis, indicating that T_{fh} cells are pro-atherogenic [23•], presumably because they induce B-cell activation and secretion of IL-21 [44,45]. One caveat to this atherosclerosis study

Table 2 Plasma membrane cholesterol and granzyme B^+, $IFN\gamma^+$, and $TNF\alpha^+$ $CD8^+$ T-cells

Model	Time prior to TCR stimulus	Plasma membrane cholesterol	Granzyme B^+ $CD8^+$	$IFN\gamma^+$ $CD8^+$	$TNF\alpha^+$ $CD8^+$
Lxrβ deficiency [30••]	-	Not reported	Not reported	↑	↑
T-cell *Acat1* deficiency [31••]	-	↑	↑	↑	↑
MβCD-cholesterol (10 µg/mL) [31••]	15 minutes	↑	↑	↑	↑
MβCD (1mM) [31••]	5 minutes	↓	↓	↓	↓
T-cell *Scap* deficiency [33•]	-	↓	Not reported	↓	↓
LDL-c (24 hours during TCR stimulus) [36]	-	Not reported	↑	↑	↑
Lovastatin (10 µM) [31••]	6 hours	Not reported	↓	↓	↓
U18666A (2 µg/mL) [31••]	6 hours	Not reported	↓	↓	↓
T-cell *Abca1/Abcg1* deficiency [26••]	-	↑	↑	↑	Not reported

T-cells were stimulated with αCD3/αCD28 for 24 h; except for T-cell *Abca1/Abcg1* deficiency where the stimulus was αCD3/IL-2 for 12 h. For *Lxrβ* deficiency and T-cell *Scap* deficiency, T-cells were stimulated by immunization in vivo

Abca1 and Abcg1, ATP binding cassette A1 and G1; *Acat1*, acyl-CoA cholesterol acyltransferase 1; *IFNγ*, interferon γ; *IL-2*, interleukin 2; *LDL*, low-density lipoprotein; *Lxrβ*, liver X receptor β; *MβCD*, methyl-β-cyclodextrin; *Scap*, sterol regulatory element-binding protein (SREBP) cleavage-activating protein; *TCR*, T-cell receptor; *TNFα*, tumor necrosis factor α

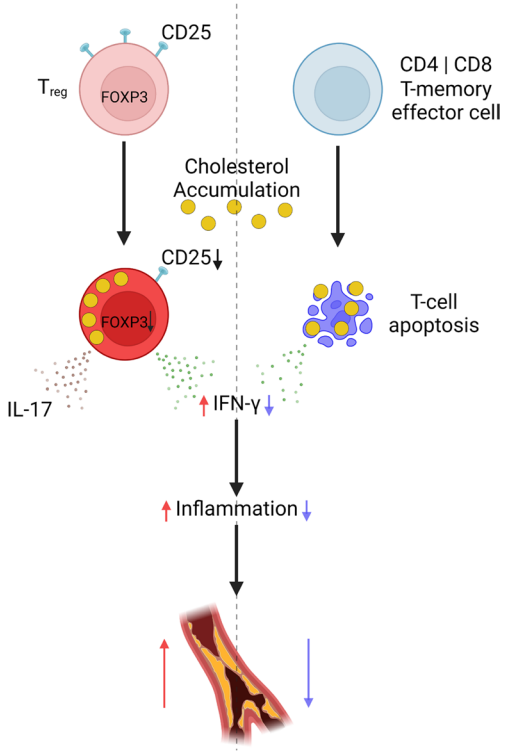

Fig. 2 Effects of cholesterol accumulation on regulatory T-cell (T_{reg}) fate and on T-cell apoptosis and downstream effects on the production of interferon γ (IFNγ), inflammation, and atherosclerosis. Created with BioRender.com

was that Bcl6 is also expressed by germinal center B-cells that have a pro-atherogenic role [44,46]. Nonetheless, this study [23•] strongly suggests that cholesterol accumulation in T_{regs} compromises T_{reg} function and enhances atherogenesis. This outcome is different from the mice with T-cell *Abcg1* deficiency that showed cholesterol accumulation and increased T_{regs}. This may be due to a higher level of membrane cholesterol accumulation in T-cells from *Apoe*$^{-/-}$ mice fed a WTD than in WTD-fed *Ldlr*$^{-/-}$ mice with T-cell *Abcg1* deficiency, simply because in the setting of T-cell *Abcg1* deficiency cholesterol esters accumulate [35], which may not have been the case in *Apoe*$^{-/-}$ mice fed WTD.

Interestingly, mice with T-cell *Abca1* deficiency show a decrease in T_{regs} [47], attributed to increased *Abcg1* expression [39], but T-cell *Abca1* deficiency is athero-protective in *Ldlr*$^{-/-}$ mice fed WTD [39]. This athero-protective effect was attributed to a decrease in membrane cholesterol accumulation due to elevated *Abcg1* expression, and a decrease in $T_{memory\ effector}$ cells that indeed may have a pro-atherogenic role [39]. In contrast, we recently found that combined T-cell *Abca1/Abcg1* deficiency decreased $T_{memory\ effector}$ cells but did not affect atherosclerosis in young *Ldlr*$^{-/-}$ mice fed WTD, while decreasing atherosclerotic plaque size in *Ldlr*$^{-/-}$ mice fed a chow diet at 1 year of age [26••]. We attributed the latter to the higher number of T-cells in plaques of *Ldlr*$^{-/-}$ mice at 1 year of age than in young mice, and thus a more prominent role of T-cells in plaque formation in aged mice [26••]. Mechanistically, T-cell *Abca1/Abcg1* deficiency increased T-cell apoptosis and, consequently, decreased IFNγ production, decreasing macrophage inflammation in lesions [26••] (Fig. 2). Even though *Apoe*$^{-/-}$ mice also show decreased T-cell IFNγ production after 20 weeks of WTD feeding, this does not compromise lesion growth [25•], presumably because pro-inflammatory effects of *Apoe* deficiency on other cell types, such as macrophages, are dominant. *Apoe*$^{-/-}$ mice fed WTD may resemble advanced CAD in humans [25•], and therefore these studies in *Apoe*$^{-/-}$ mice are most informative in providing mechanistic insights as to why T-cells in patients with advanced CAD lose their funtionality in terms of proliferation and IFNγ production, likely occurring downstream of increased T-cell apoptosis.

Conclusions and Future Directions

T-cell membrane cholesterol accumulation is key to T-cell proliferation and differentiation into cytotoxic T-cells both processes downstream of TCR signaling that are crucial to T-cell function [30••, 33•]. The exact mechanism for these findings is not yet clear. Membrane cholesterol accumulation may induce TCR clustering [31••], as such activating TCR signaling; however, studies employing artificial membranes have yielded conflicting data as to the role of membrane cholesterol in TCR signaling [48, 49], indicating that the exact mechanism remains to be elucidated.

During aging, T-cell numbers decline, and T-cells accumulate cholesterol [27, 28]. Cholesterol accumulation may induce T-cell apoptosis or T-cell exhaustion [26••, 41•], which both may contribute to the decrease in T-cell numbers.

The diminished T-cell functionality in terms of T-cell proliferation and IFNγ production in CAD patients may be the consequence of T-cell cholesterol accumulation [25•, 26••]. Similarly, cholesterol accumulation in T_{regs} of CVD patients may enhance differentiation into pro-atherogenic T-cell subsets [6, 9, 21, 22, 23•, 24] (Fig. 2). Although deficiency of T-cell cholesterol efflux pathways also increased T-cell apoptosis in atherosclerotic plaques [26••], it seems rather unlikely that high levels of cholesterol accumulation in T-cells from human atherosclerotic plaques have a similar effect. Even though the plaque environment is rich in cholesterol, plaques from human carotid endarterectomies show high numbers of T-cells [1••, 2••, 50••] that differentiate into specific T-cell subsets completely dependent on the local plaque environment [50••]. Triggers that regulate this differentiation remain to be determined. Recent single TCR sequencing studies suggest that atherosclerosis has

an auto-immune component driven by autoreactive $CD4^+$ T-cells [50••].

In conclusion, several findings, as summarized in Tables 1 and 2, indicate that T-cell membrane cholesterol accumulation is key to regulating the functionality of peripheral T-cells. This is particularly important in response to infections. Indeed, a lack of cholesterol synthesis in $CD8^+$ T-cells resulted in an attenuated clonal T-cell expansion during viral infection [33•]. Excessive cholesterol accumulation compromises T-cell functionality by inducing T-cell apoptosis [26••]. This may contribute to the increase in T-cell apoptosis and impaired T-cell functionality in patients with advanced CAD [25•].

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Declarations

Conflict of Interest V.B. receives funding from Novo Nordisk (postdoc research). The other authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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