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INVITED REVIEW

Protective and pathogenic roles of CD8$^+$ T cells in atherosclerosis

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Abstract Although infiltration of CD8$^+$ T cells in human atherosclerotic lesions has been described 30 years ago, the role of these cells in lesion development has long remained enigmatic. While experimental models hinted at their pro-atherogenic role based on circumstantial evidence, genetic mouse models of cytotoxic CD8$^+$ T cell-specific immune deficiency suggested no crucial role of these cells in lesion development. However, in recent years, more refined models of adoptive cell transfer, disruption of specific immune regulatory pathways or monoclonal antibody-mediated cell depletion have proposed both atheroprotective and pro-atherogenic functions for CD8$^+$ T cells in atherosclerosis. In particular, MHC class I-restricted CD8$^+$ T cell responses may protect from atherosclerosis, and Qa-1 restricted regulatory CD8$^+$ T cells have been defined. In addition, regulatory CD8$^+$CD25$^+$ T cells possess atheroprotective properties. However, CD8$^+$ T cells can also promote monopoiesis in hyperlipidemia, and exert prototypical cytotoxic functions to promote vascular inflammation and macrophage accumulation leading to atherosclerotic lesion development. Here, we review these findings, mostly from experimental studies that reveal a previously unrecognized complexity and important role of CD8$^+$ T cells in atherosclerosis.

Keywords Atherosclerosis · Inflammation · Lymphocytes, macrophages · T cells · CD8$^+$ T cells

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Introduction

Atherosclerosis is characterized by a chronic inflammatory process in the vascular wall that is initially triggered by lipoprotein infiltration in the vascular intima [24]. Accumulation of macrophages, which turn into foam cells upon ingestion of modified lipoprotein, is a hallmark of atherosclerotic lesion formation [13, 28, 32, 53]. Macrophages accumulate either through recruitment of their circulating precursors, i.e., monocytes, or through local proliferation in advanced plaques [13, 42]. Recently, a population of self-renewing resident arterial macrophages, which populate vessels shortly after birth and originate from CX$_3$CR1$^+$ precursors and circulating monocytes has been described, but whether it participates in macrophage accumulation during plaque formation remains to be determined [16]. Even though macrophages constitute the vast majority of immune cells in atherosclerotic lesions, it is now well recognized that adaptive immunity can modulate lesion growth [1]. Infiltration of T cells in atherosclerotic lesions in humans and animals has been demonstrated in pioneering studies [31]. Several subsets of CD4$^+$ T helper cells have subsequently been defined and their various contributions to atherosclerosis were uncovered in experimental models. Th1 polarized T cells, which produce the pro-atherogenic cytokine IFNγ, promote atherosclerosis, while immunosuppressive Foxp3$^+$ regulatory T cells limit lesion progression [1]. Th2 polarized T cells are thought to be mostly anti-atherogenic, although results are less clear-cut [1]. The role of IL-17 producing Th17 cells is still debated as various studies have produced contradictory results [1]. Although the presence of CD8$^+$ T cells in atherosclerotic lesions alongside CD4$^+$ T cells has long been documented [31], evidence pointing towards a role of these cells in atherosclerosis has long remained

circumstantial, or was regarded to not influence lesion formation. In the past few years, however, a number of studies have focused on $CD8^+$ T cells in atherosclerosis, and suggested that, similar to $CD4^+$ T cells, the role of $CD8^+$ T cells in atherogenesis is complex and subset-dependent.

$CD8^+$ T cells

Cytotoxic $CD8^+$ T cells are known as critical players in host defense. To become effector cytotoxic T lymphocytes (CTLs), naive antigen-specific $CD8^+$ T cells need to be activated by antigen-presenting cells (APCs) that present antigen on MHC class I molecules on the cell surface. Antigens are either derived from endogenous peptides (from modified self or foreign proteins), or exogenous antigens acquired by APCs (a mechanism known as cross-presentation) [30]. Killing of transformed or infected cells that present such antigens via MHCI is thought to occur through granule exocytosis and delivery of key effector molecule, the pore-forming protein perforin as well as other serine proteinases known as granzymes that then induce apoptosis of target cells. In addition, $CD8^+$ T cells produce several cytokines, such as tumour-necrosis factor (TNF)-α and interferon (IFN)-γ, that can have cytotoxic action when secreted in the vicinity of target cells [3]. Besides these classical, well established effector functions of $CD8^+$ T cells, $CD8^+$ T cells with immune suppressive functions have been described. Namely, a small subset of $CD25^+CD8^+$ T cells with immune suppressive activity has been uncovered in humans and mice [8], and a population of immune regulatory $CD8^+$ T cells that expresses a T cell receptor exclusively interacting with the MHC class Ib molecule Qa-1 has been identified in mice, with HLA-E restricted $CD8^+$ T cells being the potential equivalent in humans [25, 29].

$CD8^+$ T cells in atherosclerosis in humans

In a seminal study by Jonasson and colleagues, immunohistochemical analysis of human carotid artery plaques revealed $CD8^+$ T cell infiltration in atherosclerotic lesions [31]. We could recently corroborate abundant $CD8^+$ T cell infiltrates at various locations in atherosclerotic lesions in the carotid artery, including the plaque shoulder and the vicinity of the necrotic core [12]. Other studies have furthermore shown the presence of $CD8^+$ T cells in the vascular wall in occlusive aortic atherosclerosis [36] and in atherosclerotic lesions at various stages [55]. Notably, $CD8^+$ T cells isolated from human plaque atherectomy specimens are highly activated, much more so than plaque $CD4^+$ T cells, or T cells isolated from the blood of the same patients [22]. Abundance of $CD8^+$ T cells in the vascular intima was found to increase with atherosclerotic disease severity [21]. In vitro, $CD8^+$ T cells were unable to infiltrate early atherosclerotic lesions, whereas they efficiently infiltrated advanced human lesion tissue [21], suggesting that vascular inflammation in advanced plaques may support recruitment of circulating $CD8^+$ T cells to the vascular wall.

Associations between blood levels of specific $CD8^+$ T cell subsets and cardiovascular disease was further proposed in a study analyzing $CD8^+$ T cell proportions in frozen blood samples obtained during baseline investigations in 700 subjects in the cardiovascular arm of the Malmö Diet and Cancer Study [37]. Here, a negative correlation of the frequency of $IFN\gamma^+$ $CD8^+$ T cells among total blood $CD3^+$ T cells with the degree of carotid stenosis was found that remained significant after adjustment for major cardiovascular risk factors [37]. However, subjects in the two highest tertiles of $CD8^+$ T cells among $CD3^+$ T cells showed a trend towards an increased incidence of coronary events during the 15 years of follow-up [37]. In other studies, an expansion of $CD8^+$ T cells expressing the IL-6 receptor α chain [27] or co-expressing the inhibitory receptors PD-1 and Tim-3 [49] has been described in patients with clinical manifestations of atherosclerosis. The relevance of these findings remains to be elucidated. In particular, it is still unknown if numbers of $CD8^+$ T cells correlate with their infiltration into the vessel wall (or other organs, shown to be important for their functional contribution to atherosclerosis, as outlined below), or whether a certain cytokine or surface marker expression profile defines or contributes to the role of $CD8^+$ T cells in lesion formation.

$CD8^+$ T cells in experimental models of atherosclerosis

In murine models of atherosclerosis, $CD8^+$ T cells have been observed in atherosclerotic lesions by immunohistochemistry in *Apoe*$^{-/-}$ mice [59] or by flow cytometric analyses of vascular tissue in *Ldlr*$^{-/-}$ animals [12]. $CD8^+$ T cells can also be found in adventitial tertiary lymphoid organs in advanced atherosclerotic vessels [10, 46].

A principal role of antigen-driven T cell responses was examined in *Apoe*$^{-/-}$ mice expressing β-Galactosidase as an artificial antigen in vascular smooth muscle cells in the aorta and lung arteries. Immunized with dendritic cells presenting a β-galactosidase-derived immunogenic peptide, these mice developed strong $CD8^+$ T cell responses against cells expressing β-galactosidase, leading to non-resolving inflammation and a massive infiltration of the vascular wall with $CD8^+$ T cells and $F4/80^+$ macrophages,

resulting in larger atherosclerotic lesions than in *Apoe*$^{-/-}$ control mice [44]. These data suggested that CD8$^+$ T cells that antigen-specifically react against vascular cells promote arterial inflammation and lesion formation.

In more standard murine models of atherosclerosis, high fat diet feeding over 4 weeks in *Apoe*$^{-/-}$ mice preferentially induced CD8$^+$ rather than CD4$^+$ T cell activation in the spleen [38], and these splenic CD8$^+$ T cells produced higher levels of both IL-10 and IFNγ, suggesting that hypercholesterolemia can trigger both anti- and pro-inflammatory programs in CD8$^+$ T cells [38]. Indirect lines of evidence further suggested pro-atherogenic functions of CD8$^+$T cells: increased CD8$^+$ T cell infiltration and/or activation in inflamed vessels coincided with increased lesion development in some murine models. In mice treated with an agonist of the T cell activating co-stimulatory receptor CD137, increased CD8$^+$ T cell infiltrates in plaques were associated with enhanced lesion development [48]. Deficiency in the co-inhibitory receptor PD-1 (programmed cell death-1), which acts as an inhibitor of T cell activation, led to an infiltration of activated CD8$^+$ T cells and increased lesion formation in *Ldlr*$^{-/-}$ mice [11]. In mice with a dendritic cell-specific deficiency of TGFβRII, resulting in blunted anti-inflammatory TGFβ signaling in dendritic cells, increased CD8$^+$ T cell infiltration in plaques and augmented lesion formation were observed [41]. These data, however, present only circumstantial evidence that link CD8$^+$ T cells with atherosclerotic lesion progression. Studies more directly assessing the contribution of these cells to atherosclerosis have provided contradictory results.

Apoe$^{-/-}$*Cd8*$^{-/-}$ mice deficient in CD8$^+$ T cells display unaltered atherosclerosis

To explore the role of CD8$^+$ T cells in atherosclerosis, Elhage and colleagues analyzed atherosclerotic lesion development in CD8$^+$ T cell-deficient *Apoe*$^{-/-}$*Cd8*$^{-/-}$ mice [15]. In these mice, cytotoxic T cell responses were dramatically decreased, whereas B- as well as CD4$^+$ T lymphocyte populations and function were described to be unaltered. *Apoe*$^{-/-}$ mice lacking CD8$^+$ T cells and fed a normal chow for 18 weeks or 1 year did not show any significant changes in atherosclerotic lesion size in the aortic root and descending aorta, suggesting that CD8$^+$ T cells do not play a major role in atherosclerosis. It should be noted, however, that in this study, lesions were analyzed at two time points that represent very early lesion formation, as reflected by minimal lesions in 18 week old *Apoe*$^{-/-}$mice, and very large, advanced lesions in 1 year old mice [15]. It thus remains possible that an effect of CD8$^+$ T cells at intermediate stages of lesion development may have gone unnoticed. A possible compensation of CD8$^+$ T cell loss by other T cell subsets in CD8$^+$ T cell deficient mice was also not addressed.

MHC class I-restricted CD8$^+$ T cell responses in atherosclerosis

Endogenous peptides are displayed on the cell surface via MHC class I molecules that are recognized by cytotoxic CD8$^+$ T cells, activated by APCs [30]. Several studies have explored MHC class I-restricted CD8$^+$ T cell responses in atherosclerosis. In an early study aiming at the evaluation of the role of adaptive immunity in atherosclerosis, C57BL/6 mice deficient for surface expression of MHC Class I were employed, created by gene targeting of the β2 microglobulin locus, which are unable to mount MHC-Class I specific responses and lack cytotoxic T cells. Compared to control C57BL/6 mice, these MHCI-deficient C57BL/6 mice displayed an increased fatty streak development when fed a high fat, high cholesterol diet [18]. Although this finding suggested that MHCI-dependent antigen-presentation and the induction of CD8$^+$ T cells is anti-atherogenic, increased atherosclerosis in β2-microglobulin deficient mice may have originated from processes unrelated to CD8$^+$ T cells. For example, β2-microglobulin deficiency in mice is associated with drastically altered iron homeostasis [50] and exacerbated Toll-like receptor-dependent inflammatory responses [56].

In another study, *Apoe*$^{-/-}$ mice deficient in TAP-1 (transporter associated with antigen-processing 1), which is required for MHC class I antigen presentation, showed no alterations in plaque formation after 8 or 20 weeks of high fat diet feeding despite drastically reduced CD8$^+$ T cell levels compared to *Apoe*$^{-/-}$ controls [38]. However, in these mice, total T cell infiltration into plaques was similar in both groups, and CD4$^+$ T cell numbers were expanded in spleen, vessel draining lymph nodes and blood of *Apoe*$^{-/-}$ *Tap1*$^{-/-}$mice. Moreover, splenic CD4$^+$ T cells from *Apoe*$^{-/-}$ *Tap1*$^{-/-}$ mice isolated after 8 weeks of high fat diet feeding showed an increased proliferation when compared to controls [38]. This suggests that the loss of CD8$^+$ T cells was compensated by CD4$^+$ T cell expansion and activation in TAP-1 deficient mice.

That cross-priming of CD8$^+$ T cells may be of subordinate importance is supported by a study using *Ldlr*$^{-/-}$ mice that displayed a robust reduction in the APC-mediated cross-priming capacity due to hematopoietic deficiency in Batf3. Despite a severe reduction in OT-I CD8$^+$ T cell proliferation in response to OVA as an artificial antigen *Batf3*$^{-/-}$*Ldlr*$^{-/-}$ mice did not show any alterations in atherosclerotic lesion burden in the aortic arch and root after Wester type diet-feeding [40].

Although these studies suggest non-essential or even protective roles of CD8$^+$ T cells in atherosclerosis, the

experimental designs and potential biases induced by compensation or alterations in other cell types complicates their interpretation.

Regulatory $CD8^+CD25^+$ T cells with anti-atherogenic functions

The first description of a putative anti-atherogenic $CD8^+$ T cell subset came from a study were atherosclerosis-prone *Apoe*$^{-/-}$ mice were immunized with the ApoB-100 p210-derived peptide [9]. ApoB-100 derived peptides are considered as atherosclerosis-related antigens [34] and immunization of *Apoe*$^{-/-}$ mice with the ApoeB-100 p210 peptide has already been associated with decreased atherosclerosis [35]. Reduced atherosclerosis in mice immunized with the ApoB-100 p210 peptide was associated with an expansion of a $CD8^+CD25^+IL10^+$ T cell population in the spleen and reduced dendritic cell (DC) levels in atherosclerotic lesions. Mechanistically, $CD8^+$ T cells from p210 immunized mice had increased cytolytic activity towards DCs in vitro over $CD8^+$ T cells from the control group, an effect that was lost when $CD25^+$ cells were depleted from the $CD8^+$ T cell population. Moreover, adoptive transfer of $CD8^+$ T cells from p210-immunized mice, but not from control mice, reduced lesion development in recipient *Apoe*$^{-/-}$ mice, and this effect was dependent on the presence of $CD25^+$ cells in the transferred $CD8^+$ T cell population [9]. Recently, the same authors furthermore demonstrated that immunization with the ApoB-100 p210 peptide reduces angiotensin II-induced aortic aneurysm formation and rupture in *Apoe*$^{-/-}$ mice, an effect that was lost when $CD8^+$ T cells were depleted using an anti-CD8 monoclonal antibody [23]. In this study, $CD8^+$ T cells from p210-immunized mice had increased cytolytic activity towards angiotensin-II stimulated macrophages, and inhibited $CD4^+$ T cell differentiation into Th17 cells [23]. Altogether, these studies suggest that a population of $CD8^+$ T cells protects from vascular inflammation in the context of ApoB-100 p210 peptide immunization, possibly by reducing levels of DCs or macrophages through cytotoxic effects, by modulating $CD4^+$ T cell responses, and anti-inflammatory effects related to IL-10 expression (Fig. 1). In another study, transfer of $CD8^+CD25^+$ but not $CD8^+CD25^-$ cells sorted from non-immunized *Apoe*$^{-/-}$ mice similarly reduced lesion development in recipient mice [58]. A subset of $CD8^+CD25^+$ T cells may thus endogenously possess atheroprotective properties, in line with the description of these cells as anti-inflammatory, regulatory $CD8^+$ T cells [5] (Fig. 1). The exact mechanisms mediating the anti-atherogenic properties of this $CD8^+CD25^+$ T cell subset and the effector molecules involved remain to be determined. Interestingly, $CD8^+CD25^+$ Tregs have also been described in humans, were they represent 0.1-1% of circulating $CD8^+$ T cells [8], raising the possibility that $CD8^+CD25^+$ T cell-mediated atheroprotection may also occur in humans.

Atheroprotective Qa-1 restricted regulatory $CD8^+$ T cells and the Tfh-GC B cell axis

Although the idea of $CD8^+$ immune-suppressive T cells ($CD8^+$ Tregs) had long been met with skepticism [7], a subset of immune regulatory $CD8^+$ T cells that exclusively interact with the MHC class Ib molecule Qa-1 on target cells has been uncovered in 2004 in mice deficient for Qa-1 [25]. Subsequently, Qa-1 restricted $CD8^+$ Tregs were described to inhibit follicular helper T cells (Tfh), which express high levels of Qa-1 [33]. This process was crucial for the maintenance of self-tolerance, as genetically modified mice with a D227 K point mutation in Qa-1 (Qa-1-D227 K mice) disrupting its binding to the T cell receptor/CD8-coreceptor, and thus interfering with $CD8^+$ Treg function, developed autoimmune disease [33].

Recently, Clement et al. could first demonstrate that proportions of Qa-1-restricted $CD8^+$ Tregs were reduced in the spleen of aged *Apoe*$^{-/-}$ mice compared to young controls, while numbers of Tfh and germinal center B cells (GC B cells) were increased. Transfer of young $CD8^+$ Tregs into old *Apoe*$^{-/-}$ mice reduced splenic Tfh cell levels [10], and aged *Apoe*$^{-/-}$ mice crossed to Qa-1-D227K mice showed increased atherosclerotic lesion formation and vascular inflammation, associated with increased Tfh and GC B cell levels [10]. In these mice, depositions of IgG in atherosclerotic lesions were also increased [10].

To limit Tfh expansion in *Apoe*$^{-/-}$-Qa-1-D227 K mice, the authors targeted the co-stimulatory molecule ICOS (inducible T cell costimulator), which has a critical role in Tfh cell generation [2] by treating mice with neutralizing anti-ICOS antibodies. This rescued the pro-atherogenic phenotype observed in *Apoe*$^{-/-}$-Qa-1-D227K mice and IgG deposition in lesions, suggesting that Qa-1 restricted regulatory T cell can limit atherosclerotic lesion development through inhibition of Tfh cell-mediated activation of GC B cells [10] (Fig. 2).

Interestingly, formation of adventitial tertiary lymphoid organs (ATLOs) was accelerated in *Apoe*$^{-/-}$-Qa-1-D227 K mice, indicating that Qa-1 restricted $CD8^+$ Tregs inhibit formation of ATLO in advanced atherosclerosis [10]. ATLOs have been described in advanced atherosclerosis in mice [46] and in human atherosclerotic aneurysmal arteries [10]. The link between Qa-1 restricted $CD8^+$ regulatory T cells, ATLO formation and atherosclerotic lesion development, however, is difficult to pinpoint as ATLOs were recently proposed to be atheroprotective in advanced

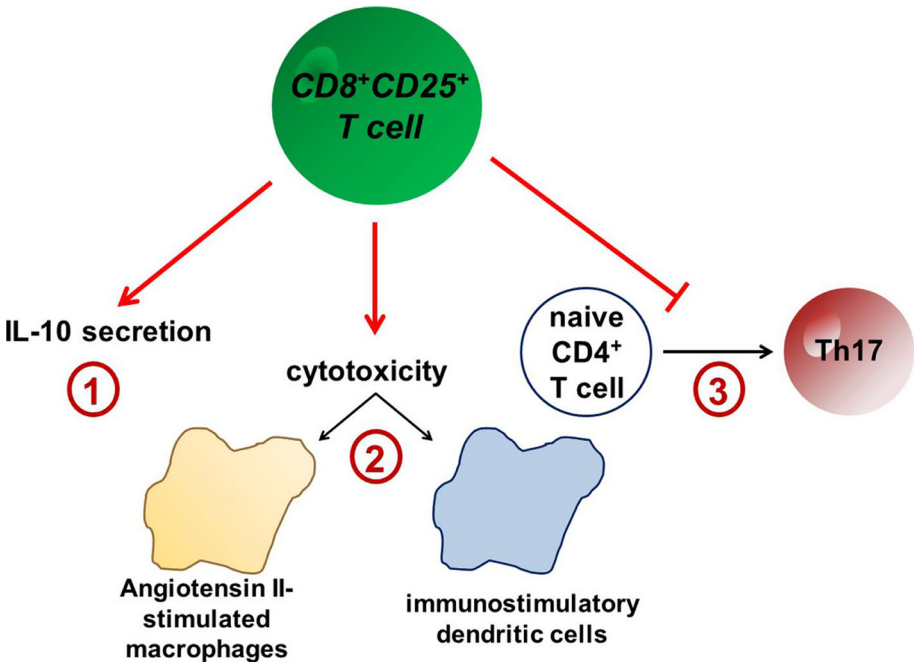

Fig. 1 Anti-atherogenic functions of $CD8^+CD25^+$ T cells. $CD8^+CD25^+$ T cells have been shown to limit vascular inflammation, atherosclerotic plaque and aortic aneurysm formation through secretion of anti-inflammatory IL-10 (*1*), cytotoxic activity towards pro-inflammatory Angiotensin-II stimulated macrophages and immunostimulatory dendritic cells (*2*), and inhibition of naive $CD4^+$ T cell differentiation into Th17 cells (*3*)

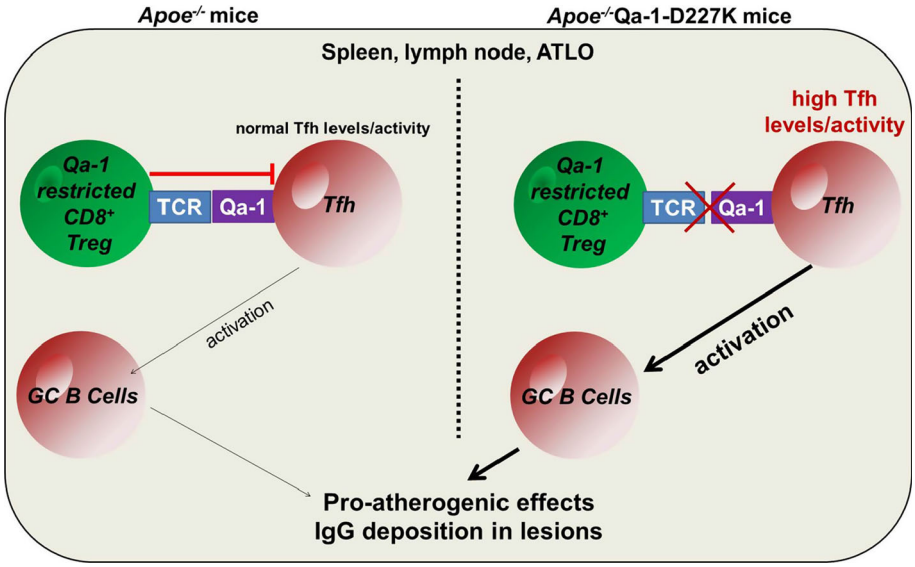

Fig. 2 Atheroprotective Qa-1-restricted $CD8^+$regulatory T cells. In lymphoid organs of hypercholesterolemic *Apoe*$^{-/-}$ mice (*left*), Qa-1 restricted $CD8^+$ regulatory T cells (Tregs) interact with Qa-1 expressing follicular helper T cell (Tfh) to inhibit their activation. This limits Tfh-mediated activation of germinal center B cells (GC B cells). When introducing a D227 K point mutation in Qa-1 that prevents its interaction with the T cell receptor (TCR) of Qa-1-restricted $CD8^+$ Tregs (*right*, *Apoe*$^{-/-}$Qa-1D227K mice), $CD8^+$ Treg-dependent Tfh inhibition is abrogated, thus leading to the accumulation and activation of Tfh, which in turn activate GC B cells, leading to increased atherosclerosis with increased IgG deposition in lesions

atherosclerosis in mice [26]. In humans, HLA-E restricted $CD8^+$ T cells have been postulated to be functionally equivalent to murine Qa-1 restricted regulatory $CD8^+$ T cells, and a defect in antigen recognition by these cells has been proposed to contribute to the development of autoimmune type 1 diabetes [29]. So far, however, no studies have investigated whether HLA-E restricted $CD8^+$ T cells may influence atherosclerosis in humans.

Pro-atherogenic effects of $CD8^+$ T cells

Recently, two studies by Kyaw et al. [39] and our own group [12] used a similar strategy of $CD8^+$ T cell depletion in atherosclerosis-prone mice using anti-CD8α or anti-CD8β monoclonal antibodies. Although this model also comes with some limitations such as the generation of antibodies neutralizing the injected antibody, it

nevertheless has been shown to efficiently allow depleting $CD8^+$ T cells in adult animals with an otherwise intact immune system, thereby circumventing biases inherent to aforementioned genetic models. In line with these considerations, both studies failed to observe any alterations in $CD4^+$ T cell levels, activation or polarization in *Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ mice after $CD8^+$ T cell depletion [12, 39], ruling out a compensation of the $CD8^+$ T cell loss by $CD4^+$ T cells. In *Apoe*$^{-/-}$ mice fed a Western diet for 8 weeks, Kyaw et al. observed reduced atherosclerotic lesion formation in mice treated with anti-CD8α or anti-CD8β monoclonal antibodies, associated with reduced macrophage accumulation in plaques [39]. We observed a similar phenotype of reduced lesion formation and macrophage accumulation in *Ldlr*$^{-/-}$ mice after 6 weeks of high fat diet feeding and weekly injections of CD8-depleting antibodies [12]. Interestingly, in both studies, levels of the chemokine MCP-1/CCL-2, which promotes monocyte infiltration into lesions [54], were decreased in vascular tissue of $CD8^+$ T cell-depleted mice, suggesting that $CD8^+$ T cells promote recruitment of monocytes into lesions. However, in adoptive transfer experiments, we could demonstrate that $CD8^+$ T cell depletion does not affect monocyte recruitment into atherosclerotic aortae.

$CD8^+$ T cells promote monopoiesis in hyperlipidemia

The accumulation of monocytes in lesions has previously been demonstrated to directly correlate with circulating monocyte levels [14]. Notably, reduced numbers of blood $Ly6C^{hi}$ monocytes were observed in $CD8^+$ T cell depleted *Ldlr*$^{-/-}$ mice [12]. $Ly6C^{hi}$ monocytes, also known as inflammatory or classical monocytes, are the murine counterparts of human $CD14^+CD16^-$ monocytes [19], and are thought to be the main circulating precursors of plaque macrophages [52]. Hence, reduced $Ly6C^{hi}$ monocyte levels after $CD8^+$ T cell depletion was suggested to secondarily cause the decrease in macrophage accumulation in lesions upon $CD8^+$ T cell depletion. As depletion of $CD8^+$ T cells was associated with reduced plasma levels of the monocyte-mobilizing chemokine CCL2, a reduced egress of medullar monocytes likely contributed to reduced circulating $Ly6C^{hi}$ monocyte levels. In addition, we could demonstrate that $CD8^+$ T cell depletion affected monocyte generation in the bone marrow, with reduced medullar levels of monocyte and late-committed monocyte progenitors (Fig. 3).

Although the mechanisms underlying $CD8^+$ T cell-mediated promotion of monocyte generation in the bone marrow during atherosclerosis remain to be elucidated, a similar property of $CD8^+$ T cells has been described in the context of acute viral infection [51]. Schurch et al. proposed that effector $CD8^+$ T cells indirectly induce proliferation and myeloid differentiation of hematopoietic progenitors during viral infection. In this model, IFNγ produced by $CD8^+$ T cells triggerd the secretion of cytokines (e.g. IL-6) by bone marrow stromal cells that in turn promoted myelopoiesis, rather than directly affecting hematopoietic progenitors [51]. In hypercholesterolemic *Ldlr*$^{-/-}$ mice, IFNγ neutralization indeed partially reproduced the phenotype triggered by $CD8^+$ T cell depletion and entailed a decrease in late-committed monocyte progenitors in the bone marrow. In addition, however, IFNγ blockade in mice also induced a retention of monocytes in the bone marrow, reflected by increased levels of medullar monocytes, while blood monocyte levels were decreased [12].

Cytotoxic $CD8^+$ T cells induce plaque cell apoptosis and inflammation

In addition, a role of prototypical cytotoxic effector molecules for the pro-atherogenic functions of $CD8^+$ T cells has been explored. In both studies of antibody-mediated $CD8^+$ T cell depletion in *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice, relative size of the necrotic core was reduced in $CD8^+$ T cell depleted mice, indicating that cytotoxic properties of $CD8^+$ T cells may contribute to their pro-atherogenic effects [12, 39]. This notion was corroborated in adoptive transfer experiment into T cell-deficient *Apoe*$^{-/-}$*Rag2*$^{-/-}$ mice, which demonstrated that $CD8^+$ T cells deficient for the cytotoxic enzymes perforin or granzyme-B lost their pro-atherogenic potential. Vascular inflammation was shown to associate with increased numbers of apoptotic cells (identified as macrophages, endothelial and smooth muscle cells) in mice receiving perforin or granzyme-B-competent cells, and it was suggested that cytotoxic $CD8^+$ T cells contribute to the genesis of apoptotic cells and necrotic cores in atherosclerotic lesions, and that macrophages are target cells for cytolyic $CD8^+$ T cells in atherosclerosis (Fig. 3).

In these same experiments, $CD8^+$ T cells lacking TNFα were furthermore discovered to not affect atherosclerotic lesion formation, whereas IFNγ-deficient $CD8^+$ T cells promoted atherosclerosis similarly to controls, suggesting that also TNFα but not IFNγ, is instrumental to $CD8^+$ T cell-induced atherosclerosis (Fig. 3). Although this study provides clear evidence that $CD8^+$ T cells exert pro-atherogenic functions by cytotoxic mechanisms and TNFα production, there are some limitations to these experiments that may preclude reaching definitive conclusions about the respective role of $CD8^+$ T cell effector molecules in atherosclerosis. $CD8^+$ T cell reconstitution was documented in the spleen but not in vascular tissue. In addition, $CD4^+$ T cell help critically influences several aspects of

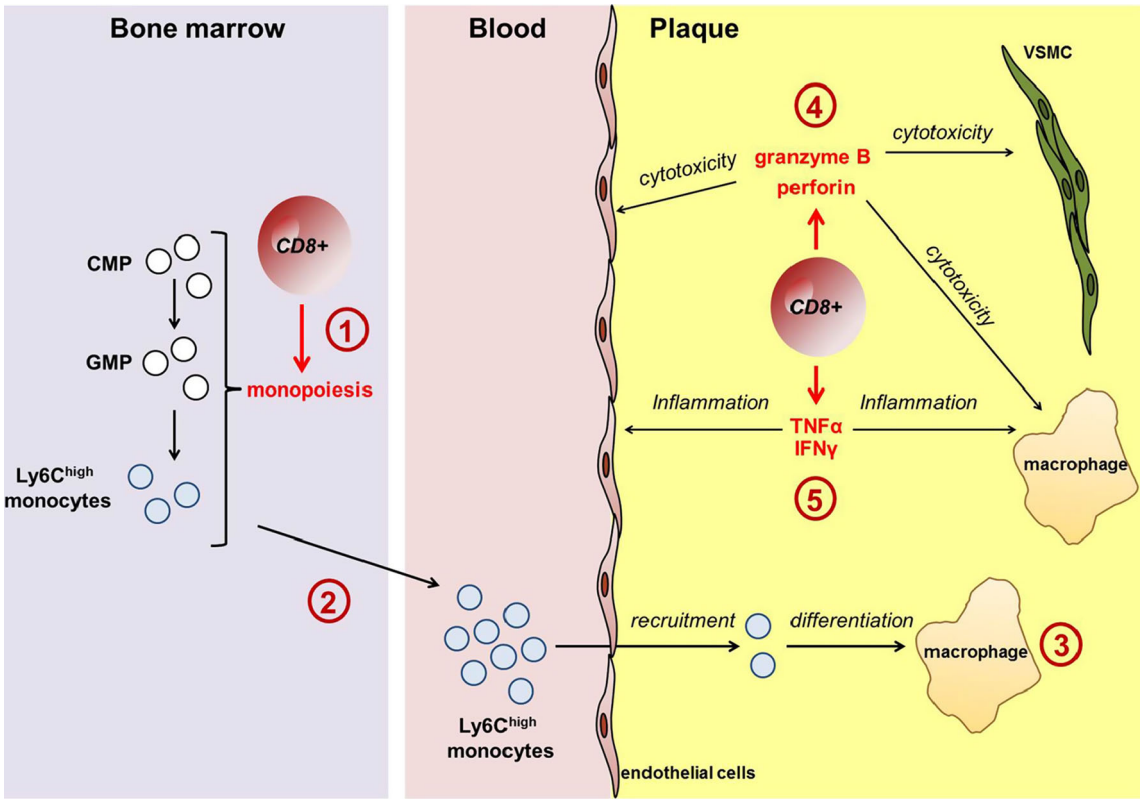

Fig. 3 Pro-atherogenic roles of $CD8^+$ T cells. In the bone marrow, $CD8^+$ T cells promote monopoiesis (*1*), thereby increasing medullar monocyte levels. This subsequently entails increased circulating levels of $Ly6C^{high}$ monocytes (*2*), the precursors of plaque macrophages, hence indirectly leading to an increased accumulation of macrophages within plaques (*3*). Within lesions, $CD8^+$ T cells secrete the cytotoxic effector molecules granzyme B and perforin, which promote death of vascular cells (*4*). $CD8^+$ T cells also secrete pro-inflammatory cytokines, such as TNFα and IFNγ that may fuel vascular inflammation through local-pro-inflammatory effects on e.g. endothelial cells or macrophages (*5*)

$CD8^+$ T cell responses [4], so that reconstitution of $CD8^+$ T cells in mice lacking $CD4^+$ T cells may have prevented full deployment of $CD8^+$ T cell functions.

$CD8^+$ T cells and infection-induced atherosclerosis

A long standing hypothesis in the field of atherosclerosis is that chronic vascular inflammation may be sustained by microbial pathogens, either through direct infection of the vascular wall and vascular cells, or through systemic effects such as induction of pro-atherogenic cytokines [6], and several studies have proposed associations of infection with specific pathogens and an increased development of atherosclerotic lesions [6]. Among those pathogens, *Chlamydia pneumoniae* has been linked with lesion formation in several clinical and experimental studies [17]. Interestingly, a study examining atherosclerotic plaques from carotid arteries showed an increased infiltration of $CD8^+$ T cells in plaques positive for *Chlamydia pneumoniae* [47]. In an experimental study, infection of C57BL/6 J mice with *Chlamydia pneumoniae* induced increased lesion formation in the aorta in response to hypercholesterolemia, an effect that was lost in $CD8^+$ T cell-deficient C57BL/6 J mice [57], suggesting a causal role of $CD8^+$ T cells in *Chlamydia pneumoniae* infection-induced acceleration of atherosclerosis. The mechanistic basis of these findings [57] remains to be explored. Given the very low level of lesion formation in these C57BL/6 J mice, experiments in atherosclerosis-prone mice should in addition be considered.

Conclusion and future perspectives

Although these recent studies begin to shed light on the complex role of $CD8^+$ T cells in atherosclerosis, further research is necessary to completely apprehend their multifaceted functions in disease. Similar to other cell populations, current evidence suggests that both atheroprotective and pro-atherogenic $CD8^+$ T cell subsets exist. $CD8^+$ T cells seem to affect atherosclerosis locally, i.e., in the plaque itself, but also systemically, with e.g. effects observed in germinal centers of lymphoid organs, ATLOs [10] or in the bone marrow [12]. Hence, uncovering

endogenous pro- and anti-atherogenic $CD8^+$ T cell subsets and studying their dynamics at various sites during atherosclerosis progression would be of interest. In addition, surface markers and effector molecules, e.g., certain cytokines that define atheroprotective versus pro-atherogenic $CD8^+$ T cell subsets relevant in atherosclerosis remain to be established. In particular, models of antibody-mediated $CD8^+$ T cell depletion that lead to an undiscriminating elimination of all CD8 expressing T cells, do not allow determining whether particular T cells subsets (e.g., activated effector T cells, memory T cells) have different effects on lesion formation. Also the mechanisms of activation of $CD8^+$ T cells in atherosclerosis are still unclear. So far, and in contrast to $CD4^+$ T cells [34], no atherosclerosis relevant antigen that may activate $CD8^+$ T cells has been described. Given the critical role of lipid metabolism in T cell function [43], hypercholesterolemia may also directly affect $CD8^+$ T cell activation. Finally, the relevance of these recent studies performed in murine models of atherosclerosis for human disease is difficult to pinpoint. For example, although potential equivalents of murine $CD8^+CD25^+$ and Qa-1 restricted regulatory T cells have been described in humans, their association with atherosclerotic disease is still unknown. Likewise, whether pro-atherogenic effector functions of $CD8^+$ T cells such as cytotoxicity towards vascular cells and cytokine-mediated pro-inflammatory effects are also relevant in human disease, remains to be determined. In addition, although models of high fat diet-induced atherosclerosis in $Ldlr^{-/-}$ or $Apoe^{-/-}$ mice recapitulate some major features of human atherosclerosis, these models suffer from numerous biases as reviewed in [20], in addition to the differences between the human and the murine immune system [45]. Findings obtained from studies using these models may thus not necessarily translate to the human situation, and additional research will be required in the future to fully address and understand the detailed mechanisms by which the different $CD8^+$ T cell subpopulations affect atherosclerotic lesion formation.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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