CD8+ T-cells in Atherosclerosis: mechanistic studies revealing a protective role in the plaque microenvironment

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The many faces of CD8$^+$ T-cells in atherosclerosis

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ABSTRACT

Purpose of review Atherosclerosis and the clinical consequence of cardiovascular disease remain the leading cause of death worldwide. Both an increase in cholesterol levels, as well as immune responses drive the pathogenesis of this disease. Although much is known about the role of many immune cell subsets in atherogenesis, research into the role of CD8$^+$ T-cells is limited.

Recent findings Both atheroprotective and atherogenic functions of CD8$^+$ T-cells have been reported. On the one hand, the inflammatory cytokines produced by CD8$^+$ T-cells exacerbate inflammatory responses, and the cytotoxic activity of these cells towards lesion-stabilizing cells such as endothelial cells drives the progression and instability of atherosclerotic lesions. On the other hand, cytotoxic activity towards antigen presenting cells and the presence of regulatory CD8$^+$ T-cell subsets dampen immunity and can limit atherosclerosis.

Summary Here we review the different roles of CD8$^+$ T-cells in atherosclerosis and discuss possible treatment strategies targeting these cells to reduce atherosclerotic lesion burden.

Keywords Atherosclerosis, Immunity, CD8$^+$ T-cells, Vaccination

1. Introduction

Atherosclerosis remains a major public health concern, as it is the leading cause of mortality worldwide. The burden of atherosclerotic cardiovascular disease is not only high in Western countries but increases fast in developing countries as well, stressing the need for adequate treatment strategies [1].

Atherosclerosis is initiated by high levels of serum cholesterol, particularly when present in low-density lipoproteins (LDL), driving infiltration of lipoproteins into the vascular intima. This, in turn, triggers an inflammatory response, resulting in the recruitment and accumulation of monocyte-derived macrophages within the vessel wall. Local activation of the macrophages results in increased cytokine production, as well as the release of enzymes and reactive oxygen species that can modify LDL to its oxidized form. The ongoing inflammation further results in the recruitment of T-cells to the lesion. Monocyte-derived dendritic cells have the capacity to take up a variety of lesion-derived antigens and travel to the lymph nodes, where they can activate T-cells. Moreover, plaque-resident antigen presenting cells can present antigen locally to the T-cells [2]. Extensive research into the role of $CD4^+$ T-cells has elucidated the role of $CD4^+$ T-cell subsets in this disease, such as the pro-inflammatory function of Th1 cells and the protective role of Tregs [3–5]. However, research into the function of $CD8^+$ T-cells in atherosclerosis lags behind, although they can play an important role in atherosclerosis initiation and progression, as they comprise a large portion of lymphocytes in both early and advanced human lesions [6, 7]. In this review, we provide a brief overview of the role of $CD8^+$ T-cells in this disease and discuss how these cells could be targeted therapeutically to reduce atherosclerotic disease burden.

2. $CD8^+$ T-cells in atherosclerosis

$CD8^+$ T-cells, also known as cytotoxic T lymphocytes, play an important role in protection against intracellular pathogens. They continuously monitor every cell in the body and are activated upon binding of their T-cell receptor (TCR) to a peptide-loaded MHC (or HLA) class I molecule. Upon recognition of a target cell, $CD8^+$ T-cells exert three main effector functions. Firstly, they secrete pro-inflammatory cytokines such as TNF-α, which can induce apoptosis, and IFN-γ, which induces upregulation of MHC-I and further promotes the inflammatory response. Secondly, Fas ligand expressed on the $CD8^+$ T-cells can bind the Fas receptor on target cells, which leads to downstream activation of the caspase cascade and subsequent apoptosis of the target cell. Finally, $CD8^+$ T-cells can release granzymes and perforin into the cleft between their own membrane and the target cell membrane, resulting in lysis of the target cell [8]. In the past few years, a number of studies have addressed the role of the different functions of $CD8^+$ T-cells in atherosclerosis (Fig. 1).

Early experimental studies using full body knockouts provided limited evidence towards a role for $CD8^+$ T-cells in atherogenesis. The introduction of $CD8^+$ T-cell deficiency in apolipoprotein E $(apoE)^{-/-}$ mice by disrupting the Antigen Peptide Trans-

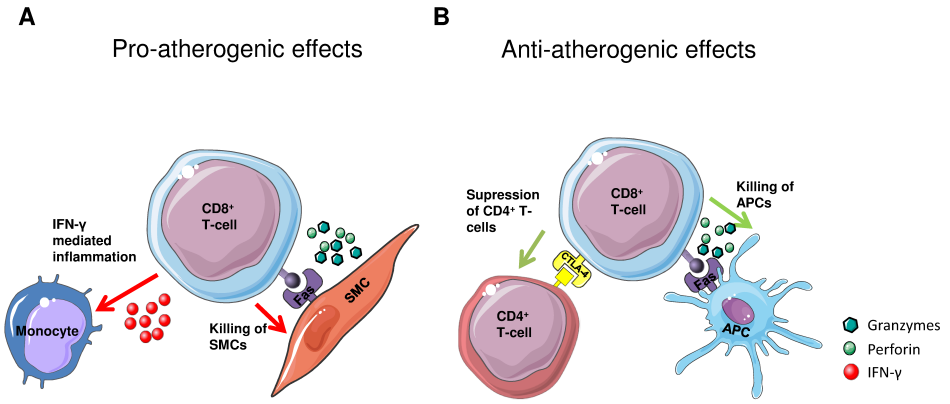

Figure 1: The pro- and anti-atherogenic effects of CD8$^+$ T-cells in atherosclerosis. (A) CD8$^+$ T-cells are reported to promote atherosclerosis via the release of inflammatory cytokines, which affects monocyte recruitment, as well as via cytolytic killing of smooth muscle cells and endothelial cells. (B) The protective functions of CD8$^+$ T-cells are mediated via suppression of CD4$^+$ T-cells by stimulating inhibitory receptors and cytolytic killing of antigen-presenting cells.

porter 1 (TAP-1)-dependent MHC class I antigen presentation, shows no difference in lesion development compared to apoE$^{-/-}$ controls [9]. Moreover, deficiency of the CD8a gene in apoE$^{-/-}$ mice does not affect the development of atherosclerosis at either 18 weeks and 1 year of age [10]. Feeding a high-fat diet to MHC-I deficient C57Bl/6 mice, which display greatly reduced numbers of CD8$^+$ T-cells as they are unable to positively select them in the thymus, does result in a large increase in plaque area compared to wild-type controls, suggesting a protective role for CD8$^+$ T-cells in atherosclerosis [11].

However, more recent studies provide a more detailed and a better mechanistic insight into CD8$^+$ T-cell function. There is a collection of work that suggests a pro-atherogenic role for CD8$^+$ T-cells in atherosclerosis. Firstly, high-fat diet fed apoE$^{-/-}$ mice show an increase in IFN-γ production by activated (CD28$^+$) CD8$^+$ T-cells in the mediastinal lymph nodes (which drain the major arteries around the heart and thus drain major lesion sites), compared to chow-fed controls, suggesting that CD8$^+$ T-cells mount a pro-inflammatory response to hypercholesterolemia [12]. However, in the splenic compartment, two distinct CD8$^+$ T-cell phenotypes were identified: a proliferative population that produces IFN-γ and a quiescent population producing IL-10, indicating that different subsets of CD8$^+$ T-cells may exert different functions. Cochain *et al.* report the presence of pro-inflammatory cytokine-producing CD8$^+$ T-cells in plaques as well as in splenic tissue [13]. Furthermore, upon depletion of these cells in initial atherosclerosis in LDL receptor (LDLr)$^{-/-}$ mice, they observe a decrease in plaque macrophage content. Interestingly, CD8$^+$ T-cells did not appear to directly affect trafficking of monocytes into the lesion. Rather, the authors suggest that the systemic absence of IFN-γ produced by CD8$^+$ T-cells contributed to reduced monopoiesis and lower levels of circulating inflammatory monocytes, resulting in the observed decrease in plaque macrophages. In contrast with this, Kyaw *et al.* do not observe a reduc-

tion in atherosclerosis development after the introduction of IFN-γ deficient $CD8^+$ T-cells compared to wild-type controls, suggesting that the role of $CD8^+$ T-cell-derived IFN-γ in the pathogenesis of atherosclerosis is limited [14]. However, they do report that the cytotoxic functions of $CD8^+$ T-cells contribute to their pro-atherogenic role. Perforin- and granzyme-B release by $CD8^+$ T-cells in early stages of lesion development in $apoE^{-/-}$ mice results in apoptosis of smooth muscle cells, macrophages, and endothelial cells, thereby destabilizing the lesion and increasing necrotic core formation.

On the other hand, a body of work has shown a protective role for $CD8^+$ T-cells in atherosclerosis. Immunization of $apoE^{-/-}$ mice with p210, an ApoB100-derived peptide, activates and expands $CD8^+$ T-cells and reduces atherosclerosis compared to controls [15]. The atheroprotective effects of the $CD8^+$ T-cells in this study are mediated by their cytolytic activity towards dendritic cells, which present ApoB100-derived peptide fragments via MHC-I molecules. More recently, these authors described a population of self-reactive $CD8^+$ T-cells to p210-derived peptides using fluorescently labeled H2Kb pentamers in $apoE^{-/-}$ mice [16]. These pentamers consist of five peptide-MHC-I complexes, which only bind to antigen-specific $CD8^+$ T-cells via recognition of the peptide presented on the pentamer by the TCR of the T-cell. Moreover, pentamer-mediated blocking of the $CD8^+$ T-cells significantly reduces the cytolytic activity of these cells, although its effect on atherosclerosis remains to be determined. $CD8^+$ T-cells are also able to confer protection against neointima formation after arterial injury by mounting a cytotoxic response against smooth muscle cells [17]. In support of that, there is a marked absence of $CD8^+$ T-cells in $apoE^{-/-}$ mice with completely ligated common carotid artery lesions (a model for neointima formation) upon Western-type diet feeding, whereas they are present in chow-fed mice [18]. This suggests a protective role of $CD8^+$ T-cells in atherosclerosis but requires further research into the mechanisms by which the Western-type diet affects the $CD8^+$ T-cells.

The contradictory role of $CD8^+$ T-cells may be explained by their heterogeneity and the various subsets that have been identified. Zhou *et al.* report the presence of a $CD8^+CD25^+$ regulatory T-cell subset in the lesions of 25-week old $apoE^{-/-}$ mice fed an atherogenic diet. These cells have a high expression of regulatory markers such as TGF-β, FoxP3, and CTLA-4 [19]. Thereby, they are able to inhibit the proliferation of $CD4^+CD25^-$ T-cells and the cytolytic activity of $CD8^+CD25^-$ T-cells. As anticipated, adoptive transfer of $CD8^+CD25^+$ T-cells reduces atherosclerosis development. Clement *et al.* report a protective role for Qa1-restricted $CD8^+CD44^{high}CD122^+$ regulatory T-cells in the development of atherosclerosis in $apoE^{-/-}$ mice [20]. These $CD8^+$ Tregs interact via their TCR with the Qa1 antigen-presenting molecules (the murine variant of HLA-E) on T follicular helper cells and inhibit their function. However, as the lesions advance, the numbers of these regulatory cells decrease, associated with an increase in T follicular helper cells. These cells, in turn, stimulate the expansion of germinal center B cells, tertiary lymphoid organ formation and increase immunoglobulin production, resulting in an increase in atherosclerosis. More recently, a study in $LDLr^{-/-}Stat4^{-/-}$ mice reported a similar phenotype, characterized by a reduction in T follicular helper cells and plasma B cells associated with an increase in $CD8^+CXCR5^+CD275^+CD122^+$ T reg-

ulatory cells. Besides the effects on CD8$^+$ Treg generation, STAT4 deficiency also affects macrophages by altering the cytokine profile of these cells, further promoting CD8$^+$ Treg development [21].

In human atherosclerosis, CD8$^+$ T-cells also make up a large proportion of the lymphocytes present in the lesion and are mainly found in the shoulder regions and fibrous caps [6, 7, 22]. Moreover, the CD8$^+$ T-cell numbers in blood correlate with the incidence of coronary events [23]. Naïve CD8$^+$ (CCR7$^+$CD45RA$^+$) T-cells are present in higher numbers in patients without significant coronary disease, whereas they are decreased in patients with more advanced atherosclerosis, suggesting activated CD8$^+$ T-cells have a pro-atherogenic effect [24]. In support of this, there is a negative correlation between these naïve CD8$^+$ T-cells and pulse wave velocity, a well-documented risk factor of coronary artery disease, thus implying that higher numbers of differentiated CD8$^+$ T-cells may augment atherosclerosis. In particular, expression of the IL-6 receptor α chain (IL-6Rα) correlates with pathogenicity of the CD8$^+$ T-cells [25]. Expression of the IL-6Rα on CD8$^+$ T-cells in the circulation is decreased in patients suffering from coronary artery disease, which is associated with an increase in CD8$^+$ effector T-cells that express granzyme B and the pro-inflammatory transcription factor T-bet, indicating a pathogenic role for CD8$^+$ T-cells in coronary artery disease. However, similar to murine models, different CD8$^+$ T-cell subsets appear to exert different functions in humans. Whereas a high percentage of CD8$^+$CD25$^+$ T-cells shows a correlation with a high degree of carotid stenosis, CD8$^+$CD56$^-$IFN-γ+ T-cells are associated with less carotid stenosis [23]. Qiu *et al.* describe a subset of CD8$^+$ T-cells expressing both PD-1 and Tim-3, which is increased in human atherosclerotic patients compared to controls [26]. This subset is associated with decreased production of pro-atherogenic cytokines and an associated increase in anti-atherogenic cytokines.

3. Therapeutic outlook on treatment strategies targeting CD8$^+$ T-cells

Overall, these studies show that CD8$^+$ T-cells play an intricate role in atherosclerosis, but targeting the right subsets may provide new avenues for treatment. An interesting therapeutic approach that may prove useful to induce protective CD8$^+$ T-cells, is vaccination. Several studies have already demonstrated the atheroprotective potential of vaccination using LDL-derived peptides via induction of CD4$^+$ regulatory T-cells [27–29]. As mentioned above, vaccination with p210 can induce a CD8$^+$ T-cell population that can lyse dendritic cells and reduce lesion formation [16, 17]. Moreover, p210 vaccination is also reported to be protective against aortic aneurysm formation by boosting CD8$^+$ T-cell responses that have cytolytic activity against macrophages and via reducing the polarization of CD4$^+$ T-cells into the Th17 phenotype [30]. Furthermore, vaccination using BSA conjugated to the ApoB100-derived p2 and p45 peptides, but not the p210 peptide, induces a significant increase in CD8$^+$CD25$^+$FoxP3$^+$ and CD8$^+$CD25$^-$FoxP3$^+$ regulatory T-cells when compared to controls [29]. Inducing CD8$^+$ T-cell responses against vascular endothelial growth factor receptor 2 and CD99 (a membrane protein involved in transmigration of monocytes through endothelial

cells) using DNA vaccinations are also proven to be effective in reducing atherosclerosis development [31, 32]. Taken together, these studies suggest that boosting $CD8^+$ T-cell responses in atherosclerosis holds promise as a treatment against atherosclerosis. However, some challenges remain to be met before an optimal vaccine can be designed. Although promising results have been obtained with vaccination against LDL-derived antigens, the epitope against which $CD8^+$ T-cell responses are directed in atherosclerosis remains to be determined [33]. Furthermore, it is of vital importance to identify the optimal adjuvant, as several studies have reported that adjuvants alone can affect atherosclerosis development [34–36]. Finally, one must be careful to induce only protective $CD8^+$ T-cell responses and avoid activation of the pro-atherogenic $CD8^+$ T-cell subsets discussed above.

Other potential treatment strategies in that could boost protective $CD8^+$ T-cell responses include TCR stimulation and administration of IL-2 complexes. Stimulation of the TCR using modified anti-CD3 antibodies induces regulatory $CD8^+$ T-cells which can inhibit antigen-specific $CD4^+$ T-cell responses in several *in vitro* models [37, 38]. Although oral of administration of anti-CD3 in a murine model was shown to reduce atherosclerosis development by the induction of $CD4^+$ regulatory T-cells, no effects on $CD8^+$ T-cells were reported [39]. Similarly, intravenous administration of anti-CD3 in $LDLr^{-/-}$ mice reduces atherosclerosis via increased $CD4^+$ regulatory T-cell responses, and a significant decrease in aortic CD8 mRNA levels was observed, although no $CD8^+$ T-cell subsets were investigated in this work [40]. Immune complexes of IL-2 with anti-IL-2 monoclonal antibodies can also activate and expand $CD8^+$ T-cell populations [41, 42]. Different IL-2 complexes can be used to stimulate $CD4^+$ regulatory T-cells or $CD8^+$ T-cells, based on their binding affinity to CD25 [43]. Thus far, several studies have shown the protective effect of inducing $CD4^+$ Tregs in atherosclerosis using IL-2 complexes [44–46], but to our knowledge, the effect of IL-2 complexes on $CD8^+$ T-cells has not been studied in this disease. Therefore, there is a need for research that sheds more light on the effects of TCR stimulation and IL-2 complex therapy on $CD8^+$ T-cells in atherosclerosis to investigate the clinical potential of this treatment.

On the other hand, disabling atherogenic $CD8^+$ T-cell subsets may prove to be effective as well. MHC-I peptide multimers are commonly used to detect antigen-specific $CD8^+$ T-cells [47, 48]. Coupling peptide-MHC complexes to nanoparticles can be used to target antigen-specific $CD8^+$ T-cells [49–51], which opens up avenues for specific killing of autoreactive $CD8^+$ T-cells in atherosclerosis. DNA vaccination in a type 1 diabetes model against proinsulin, towards which the $CD8^+$ T-cell response is directed in this disease, results in a deletion of $CD8^+$ T-cells that are reactive to proinsulin [52]. This is likely mediated via increased apoptosis of $CD8^+$ T-cells due to a reduction in costimulatory signals, or via active suppression by regulatory T-cells. Once antigens against which $CD8^+$ T-cells are directed in atherosclerosis have been identified, a similar vaccination strategy may prove useful to delete pro-atherogenic $CD8^+$ T-cell subsets.

Finally, current treatment of atherosclerosis focuses mainly on lipid-lowering strategies by lifestyle changes and the use of statins. However, statin treatment results in a 25% reduction in cardiovascular disease events [53], stressing the need for new therapies that target the underlying immunologic pathogenic mechanisms as well. Of note, choles-

terol lowering in human cells, as well as murine models, has been reported to reduce CD8$^+$ T-cell proliferation, due to reduced lipid raft formation and TCR signaling [54]. Indeed, inhibiting cholesterol esterification in T-cells enhances proliferation and effector function of CD8$^+$ T-cells, but not CD4$^+$ T-cells, in a murine cancer model [55]. In this study, cholesterol esterification is inhibited via knockout of Acyl-CoA cholesterol acyltransferase-1, as well as pharmacological inhibition of this enzyme using avasimibe, which was in clinical trials as a drug against atherosclerosis development. The commercially available statins simvastatin and pitavastatin are reported to reduce the proliferative response and expression of IFN-γ in human T-cells [56]. Furthermore, atorvastatin and pravastatin treatment in virologically suppressed HIV-infected persons also reduces CD8$^+$ T-cell proliferation and activation, but also down-regulates markers of CD8$^+$ T-cell exhaustion [57]. Finally, statin treatment reduces the number of CD8$^+$ T-cells in the blood of patients with Hashimoto's thyroiditis, and *in vitro* experiments show that Pravastatin, Mevastatin, Cerivastatin, and Simvastatin are able to induce CD8$^+$ T-cell apoptosis [58]. Thus, statin treatment may impair CD8$^+$ T-cell function in cardiovascular disease patients, which may affect the pathogenesis of the disease depending on which subsets are most affected. This stresses the need for a better understanding of the effects of current and future therapeutics on the CD8$^+$ T-cell response in atherosclerosis.

4. Conclusion

Studies in both humans and in murine models have identified an important role for CD8$^+$ T-cells in atherosclerosis development and progression. Different CD8$^+$ T-cell subsets are present in atherosclerotic lesions and can either augment or limit lesion development. The discovery of the protective functions of CD8$^+$ T-cells is highly relevant for the development of new treatments for atherosclerosis, as methods to induce protective CD8$^+$ T-cells, such as vaccination, can be considered as possible therapies. However, future studies will need to show that no pro-atherogenic functions of CD8$^+$ T-cells are boosted as well before these therapies can be considered as treatments in human patients.

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