T CELLS ARE ACTIVE PARTICIPANTS IN THE PROGRESSION OF Atherosclerotic Plaques

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Atherosclerosis is an inflammatory disorder that affects the arterial blood vessels. Even if the pathogenesis of atherosclerosis is not completely elucidated, it is generally accepted that the adaptive and innate immune systems are involved in every phase of the atherosclerotic process. Among the immune cells that accumulate in atherosclerotic lesion, T cells subtypes perform important and varied duties during the development of the plaque. This review summarizes the roles played by T cell subpopulations in the pathogenesis of atherosclerosis.

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1. Introduction

Atherosclerosis, characterized by the accumulation of lipids and fibrous elements in subendothelial space, is the main cause of myocardial infarction and ischemic stroke [1]. Lipid accumulation is a stimulus for immune cells influx into the thickened intimal area which leads with time to atherosclerotic lesions that in turn can grow large enough to narrow or block the arterial lumen [2]. Mature atherosclerotic plaques may cause clinical complications such as stenosis, followed by the rupture of the plaque, which exposes the prothrombotic material in the plaque to the blood followed by sudden thrombotic occlusion of the artery at the site of disruption. In the heart, the presence of atherosclerotic disease may lead to myocardial infarction and heart failure, whereas in the major arteries, that supply the brain, it can cause transient ischemic attacks or stroke. When atherosclerosis affects the arteries, which conduct the blood to the other parts of the body, it can cause the renal impairment, hypertension, mesenteric ischemia, intestinal ischemia, abdominal aortic aneurysms and limb ischemia [3]. It is well documented that the immune system, which includes innate and adaptive immunity, plays an important role in the development of atherosclerosis from the initiation stage to thrombotic complications. However, many questions remain to be answered about the mechanisms by which immune cells can enhance or reduce the atherosclerotic process.

2. Lipids and immune cells build up the atherosclerotic plaques

Atherosclerosis is a continuous and progressive disorder of arteries and can be characterized through a pre-lesional stage, which imply a lipid disorder and a critical inflammatory process, and a lesional stage marked by the complex plaque formation [4]. Human atherosclerotic plaques are localized in the arterial intima and include different cell types such as immune cells (monocytes/macrophages, T cells, B cells, NK cells, dendritic cells and mast cells), migrated smooth muscle cells (SMCs), lipids and debris embedded in hyperplastic extracellular matrix [5].

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The development of human atherosclerotic lesions begins with diffuse arterial intimal thickening and progresses to fatty streak lesions, atherosclerotic plaques, and then advanced complicated lesions [6]. Intima thickening is due to the progressive accumulation of modified and reassembled lipoproteins (MRLs) and immune cells, mainly monocytes/macrophages and T cells [7]. The next step that contributes to the development of atherosclerotic plaque is given by the lipid-laden macrophages, known as foam cells, which are considered the hallmark of the fatty streaks [8]. In the late stages of atherosclerosis even endothelial cells take up modified lipids becoming endothelial derived foam cells [9]. Fatty streaks do not cause symptoms and are prevalent in young individuals. These nascent plaques also contain T cells and can progress into mature atherosclerotic plaques or disappear with time [3] (Hansson and Libby 2006). Mature plaques are characterized by the accumulation of additional inflammatory cell subsets (B cell, natural killer T cells, dendritic cells and mast cells) and modified lipids (oxidized LDL) that form a core region surrounded by a cap of SMCs and a collagen-rich matrix [10]. Accumulated data revealed that many of these immune cells become activated and produce growth factors, chemokines and cytokines [11].

### 3. Diversity of T cells involved in atherosclerotic process

T cells represent an important component of both early and late atherosclerotic lesion. The T cells form a very heterogenic group classified according to their functions and surface markers. The highest functional heterogeneity of T cells is related to the expression of certain cell surface receptors. Some of these receptors are expressed only in certain stages of differentiation or activation for short periods, while others are permanently active and mark the type of lymphocyte. Different T cells surface receptors such as i) receptors for antigen recognition (T cell receptors, CD3 complex and CD4 or CD8 coreceptors), ii) costimulatory molecules (CD28, CD154, CD40L) and iii) adhesion molecules (CD2, CD11/CD18) participate in the signaling that regulates the adaptive immune response. T cells receptors cannot recognize and bind antigens directly. In that case, the antigen recognition is initiated by molecular interaction between the T cells receptor (TCR) and a complex of peptide antigen with major histocompatibility complex (MHC) molecules that are expressed on the surface of antigen presented cells (APCs). Based on their interaction with the MHC molecules, T cells are divided into two subsets: CD4+ T and CD8+ T cells. CD4+ T cells, known as helper T (Th) cells, bind antigen in association with MHC II molecules. The main function of Th cells is to assist other cells of the immune system such as B cells, phagocytic cell and CD8+ T cells to perform their immune functions. CD8+ T cells which recognize antigens loaded on MHC class I molecules are cytotoxic T lymphocytes (CTLs). These cells contribute to resistance against intracellular infections by killing target cells trough releasing toxins [13]. Naive T cells differentiate further into distinct cell populations with different functions after antigen stimulation. Studies to date have revealed some of T cell subsets and populations involved in atherosclerotic process which proves the important role played by adaptive immunity in cardiovascular diseases. The temporal and functional sequence of events, types of T cells and receptors that stimulate the building up of the lipid deposits is of high significance in the understanding of the pathogenesis of atherosclerosis.

### 4. T helper cells, amplify the local inflammatory response in atherosclerosis

CD4+ T cells are more prominent participants than CD8+ cells in human atherosclerotic plaque [11]. From the functional point of view, CD4+ T cells can be classified into CD4+ effector T cells and CD4+ regulatory T cells [14] as shown in Fig. 1.
Fig. 1. Possible differentiation of CD4+ T cells in atherosclerosis suggested by the present data. APC: antigen presenting cells; Th: T helper; Treg: regulatory T cells; IFN: interferon; IL: interleukin; TGF: transforming growth factor.

Most CD4+ effector T cells detected in atherosclerotic lesions express the αβTCR receptor and have a T-helper 1 (Th1) phenotype. Th1 cells are derived from naive CD4+ T cells precursor after the antigen stimulation and costimulating activation in the presence of certain cytokines such as IL-12 and interferon-γ (IFN-γ). The defining characteristic of Th1 cells is their production of IFN-γ, a pro-inflammatory cytokine that activates mainly macrophages [15]. Besides IFN-γ, Th1 releases IL-2, IL-3, tumor necrosis factor (TNF) α and β, which amplify the inflammatory response [16]. The predominance of Th1 cells in atherosclerosis is described by several studies, which showed that many CD4+ cells and large amounts of IFN-γ collocate in human and mouse atherosclerotic plaques. Secretion of IFN-γ leads to several processes such as the recruitment of T cells and macrophages in the plaque, the foam cells formation by enhancing the uptake process of lipids by macrophages and the increase of the number of antigen presenting cells (APC). Also, IFN-γ facilitates the production of IgG2a antibodies by B cells and enhances the secretion of Th1-promoting cytokines, which continue to drive the pathogenic process that once started, appears to trigger a cascade of proatherogenic processes [17, 18]. T-helper 2 (Th2) differ from Th1 in that they secrete anti-inflammatory cytokines such as IL-4, IL-5, IL-10, and IL-13 and induce B and B-1 cells to produce IgG1a and protective IgM antibodies, respectively [10]. It was noticed that the production of antibodies play a key role in atherosclerotic process. Thus, the synthesis of IgG1 antibodies points a predominant Th2 response while IgG2a is the indicative of Th1 responses [19]. Several studies showed that Th2 cytokines could both attenuate and promote atherosclerosis. The cytokines secreted by Th1 and Th2 cells have inhibitory effect upon the differentiation and maturation of one over the other, which means that in the atherosclerotic lesion exist a cross regulation between these subtypes of T cells. Therefore, IFN-γ and IL-12 suppress Th2 responses whereas IL-4 and IL-10 inhibit Th1 pathway and cytokine secretion [17, 20]. In consequence, many studies suggest that Th1/Th2 imbalance plays an important role in the development of atherosclerosis and plaque rupture. The third subtype of CD4+ effector T cells which is supposed to be implicated in atherosclerotic lesions is Th17. These cells represent a population of T cells recently described, characterized by the production of pro-inflammatory molecules belonging to IL-17 cytokine family that includes the founder molecule IL-17 (or IL-17A), IL-17B, IL-17C, IL-17 D, IL-17E (or IL-25), and IL-17F [14]. Reported data have shown that IL-17A and IL-17F are responsible for the release of some pro-inflammatory cytokines and chemokines. They can regulate angiogenesis and are also involved in the synthesis of matrix metalloproteinases and C-reactive protein. Therefore, it was assumed that Th17 cells might play a significant role in atherosclerotic plaque destabilization. de Boer et al. noticed that different IL-17 family members were present in atherosclerotic plaques. However it was shown that pro-inflammatory cytokines IL-17A and IL-17F were rather released by mast cells and neutrophils than the Th17 cells. In
addition, IL-17E, an anti-inflammatory cytokine, was found in both normal and atherosclerotic vessels, where can take part in the regulatory process of vascular inflammation [21].

5. The protective activity of regulatory T cells in atherosclerosis

Several in vitro experimental studies and those performed on patients with atherosclerosis showed the antiatherogenic and atherosclerotic plaque stabilization effects of IL-10. TGF-β another anti-inflammatory cytokine, also secreted by T cells was found to possess antiatherogenic properties [18]. It is also known that the major cellular source that produces IL-10 and TGF-β is represented by regulatory T cells (Treg), thus suggesting their active role in the control of atherosclerotic process. Treg cells have a CD4+CD25+CD127low phenotype and are characterized by the expression of transcription factor FoxP3, which has a crucial role in their suppression function [15]. The development and maturation of Tregs can be achieved both within the thymus and in the periphery. Regulatory T cells generated within the thymus are named naturally Tregs (nTreg) and they exist in the absence of antigenic stimulation. The important role of natural Tregs is to induce and maintain immune tolerance [22]. In the periphery, Tregs are induced from naive CD4+ T cells during an active immune response and the existence of a specific cytokine environment and they are called adaptive or induced Tregs (iTreg). Therefore, naive CD4+CD25- cells are converted in the presence of IL-10 and TGF – β into regulatory T cells type 1 (Tr 1), T helper 3 (Th3) regulatory T cells respectively [23]. Recent data suggest that Tregs have the capacity to suppress both pathogenic Th1 and Th2 responses, suggesting their important protective role against atherogenesis. Tregs proved to be protective in murine atherogenesis and to reduce plaque progression in atherosclerotic animals [24, 25]. The presence of Tregs in human atherosclerotic lesions was found to be reduced but they were present in all developmental stages of atherosclerotic plaque [26]. More than that CD4+CD25+Tregs may exert their suppression role on macrophage foam-cell formation and can redirect the macrophage differentiation toward an anti-inflammatory cytokine producing phenotype [27]. In the well-established Apo E-deficient murine model of atherosclerosis, Treg number and the expression of Treg related mediators were much lower compared with age-matched C57BL/6J mice. This study also demonstrated that Th17 related mediators which increased markedly and achieved their maximum expression values at early stage (8–16 weeks of age) in ApoE−/− mice may promote the development of atherosclerotic process. In consequence, the authors proposed the existence of Th17/Treg functional imbalance during atherogenesis in ApoE−/− mice [28].

All these new findings underscore the importance of Tregs in the slow down of the inflammatory process building up in the arterial wall. The control of differentiation of these cells and their function may have a high potential to be used in the future as therapeutic targets for prevention of atherosclerosis.

6. Cytotoxic T cells populate the advanced lesions

There are few studies evaluating the role of cytotoxic T cells (also known as cytolytic T cells, CTLs or CD8+ T cells) in atherosclerosis. The data demonstrated that on the arterial intima and the adventitia of patients with symptomatic atherosclerosis had a higher number of CD8+ T cells than the arterial media. In addition, the increased number of intimal CD8+ T cells was directly correlated with the severity of atherosclerotic lesions. There were also reports showing that the arterial adventitia and the intima in arterial sectors are affected by advanced atherosclerotic lesions being freely accessible for effector CTLs [29]. Studies performed in ApoE−/− mice showed that CD8+ T cells are able to promote the formation of an atherosclerotic lesion when their stimulation was induced by the expression of a foreign antigen in the vascular wall. [30]. After activation, CD8+ T cells secrete large amounts of gramine B, a serine protease, by which cytotoxic cells may accelerate vascular smooth muscle cell apoptosis and plaque destabilization in atherosclerotic lesions [18]. Further studies regarding the role of CTLs in the progression and vulnerability of atherosclerotic plaques are desirable for a better understanding of atherosclerosis.
7. The dual function of natural killer T cells in atherosclerosis

Another class of lymphocytes identified in atherosclerotic lesions is represented by natural killer T cells (NKT). These cells are a subset of mature unconventional T cells that co-express a semi-invariant T cell receptor (Vα14-Jα18 α-chain paired with a Vβ8 or Vβ2 β-chain) and markers characteristic for NK cells such as CD161 (NK1.1), CD122 (IL-2Rβ) and CD56. The T cell receptors located on NKT cells recognize lipids and glycolipids presented by MHC class I like molecules CD1d, which are expressed on most antigen presenting cells [31, 32]. NKT cells are a thymus-dependent T-cell subset, different from CD4+ and CD8+ T cells, but able to produce large amounts of both pro- and anti-inflammatory cytokines. Thus, they can enhance microbial immunity and tumor rejection, suppress autoimmune disease and promote tolerance. However, the activity of these cells may also be unfavorable for the host since they may aggravate atherosclerosis, allergic responses and some autoimmune diseases. In humans, this class of NKT lymphocytes include CD4+, double negative (DN; CD4–CD8–) and CD8+ NKT cells, while in mice only the first two subsets has been identified [33]. Tupin et al. revealed the implication of NKT cells in the development of atherosclerosis for the first time. The study showed that CD1d deficiency reduces atherosclerosis, whereas the administration of α-galactosylceramide (α-GalCer), a synthetic glycolipid that activates NKT cells via CD1d, induced a 50% increase in lesion size in ApoE−/− mice [34]. NKT cells were also identified in human atherosclerotic lesions where they accumulate in rupture-prone shoulders of atherosclerotic plaques [31, 35]. The data showed that in ApoE−/− mice, CD4+ NKT cells present proatherogenic activity, whereas the DN NKT cells seem to have a limited proatherogenic potential due to their higher expression of Ly49 inhibitory receptors [36]. Further, Puijvelde et al. demonstrated that in hyperlipidemic conditions, the activation of NKT cells via α-GalCer induced a reduction of lesion formation in LDLR+/- mice [32]. All this new data propose that NKT cell subsets can regulate in different ways the immune mechanism of atherosclerosis.

8. Concluding remarks

The complexity of adaptive immune system results from the numerous specialized effector T cells that are able to perform various tasks which enhanced or by contrast regulate the inflammatory process. The accumulated results suggest that during the development of atherosclerosis, not only the monocytes but also naive lymphocytes pass through the arterial endothelial layer where they start the differentiation into T cell subsets. Each subset releases distinct cytokines, which seem to be engaged in the plasticity process of effector T cells and can influence the behavior of other cells present in the atherosclerotic lesion. Most of the available data, describe the capacity of Th1 – type cytokines to augment atherosclerosis while cytokines release by Treg decreases lesions size and inflammation. The role played by Th2 and Th17 in atherosclerotic plaque requires further investigations since divergent results were found under different experimental conditions. In addition, the balance of T cells subsets has revealed to be very important in the pathophysiology of atherosclerosis. NKT cells which have features of both NK cells and conventional T cells seem to participate in the first stages of atherosclerosis due to their T-cells receptors which are able to recognize lipid antigens presented by CD1. CD8+ T cells, although fewer than CD4+ T cells, are able to accelerate formation and destabilization of atherosclerotic lesion. Further studies are necessary to elucidate whether in the chronic inflammatory process of atherosclerosis are involved more types of T effector cells and which are the mechanisms involved. Therefore, future therapeutic strategies must act on different T cells subsets in a selective manner to correct the imbalances that may be caused by risk factors in the immune responses generated by these cells, during the progression of atherosclerosis.

References