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Editorial

Regulatory T cells getting to the heart of the matter

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Inflammation in the arterial intima is a key pathogenic mechanism in atherosclerosis and development of acute cardiovascular events such as myocardial infarction (MI) and stroke [1]. The major cause of this inflammation is accumulation low density lipoprotein (LDL) particles in the arterial extracellular matrix where these particles become oxidized and release pro-inflammatory lipid metabolites. The vascular inflammatory response may initially help to clear the oxidized LDL but continuous exposure to oxidized LDL will lead to activation of fibrous repair processes and development of atherosclerotic plaques. If plaque inflammation in not properly resolved the fibrous components become degraded increasing the risk for plaque rupture and thrombotic occlusion of the artery.

Interestingly, there is accumulating experimental evidence that the adaptive immune system plays an important role in modulating inflammatory activity in atherosclerotic plaques [2]. The presence of activated T cells in human atherosclerotic plaques was first reported by Jonasson et al [3] in 1986 suggesting involvement of either autoimmune responses against plaque components or the presence of infectious microorganisms. Subsequent studies have confirmed that both may occur but the most important appears to be activation of autoimmune responses against oxidized LDL. There are even reports of pro-inflammatory T cells targeting apparently normal LDL [4]. Studies performed in mouse models of atherosclerosis suggest a complex

interaction between adaptive immunity and the plaque [5].Th1-type T cells has been shown to drive plaque inflammation as well as plaque growth and destabilization. Th2 and Th17 T cells can have both pro-inflammatory and stabilizing effects depending on the environment, while regulatory T cells (Tregs) have been shown to have a protective role. The balance between the activation of these T cell subsets will determine if the atherosclerotic disease will stabilize or progress.

Tregs are immune-inhibitory cells that protects against autoimmunity by suppressing immune responses to self-antigens as well as pathogenic immunity against foreign antigens such as allergens and dietary antigens [6]. They are characterized by expression of the transcription factor FoxP3 and are formed either in the thymus (natural Tregs) or in the periphery (induced Tregs). The most important suppressive mechanisms used by Tregs are depletion of local IL-2 supplies (which T effector cells require to proliferate) through a high cell surface expression of the IL-2 receptor CD25 and release of anti-inflammatory cytokines such as IL-10 and TGF-β. Atherosclerotic plaques contains low numbers of Tregs as compared to other chronically inflamed tissues [7] suggesting that local tolerance may be impaired. The concept that Tregs have an athero-protective function is based on experimental animal studies in which depletion of Tregs or Treg cytokines has been found to aggravate the disease [2]. These observations have raised hope that Tregs could be used to reduce stabilize

high risk atherosclerotic lesions. However, since studies of Tregs and atherosclerosis almost exclusively have been performed in animal models of the disease it has remained to be clarified if these processes are of any clinical importance. Against this background the paper from Hasib and co-workers in the present volume of *Journal of Internal Medicine* is of considerable interest. They demonstrate that patients suffering from acute MI are characterized by both reduced Tregs frequencies and function. Moreover, they show that this is true not only in the acute phase but also during a 12-months follow-up period. This is important because previous reports demonstrating reduced number of Tregs in patients with acute coronary syndromes have not been able to clarify whether this represents a response to on-going tissue injury (for example by recruitment of circulating Tregs to injured tissue) or a more permanent characteristic of coronary disease patients. The study by Hasib and co-workers also adds to our understanding of the role of Tregs in coronary disease patients through the extensive Treg phenotyping performed. The identification of Tregs in mice by flow cytometry is relatively simple and requires only analysis of CD25 and FoxP3 expression. In humans living in an environment where the immune system is continuously challenged by foreign antigens this is considerably more difficult. To overcome this problem Hasib and co-workers used several different markers to identify Tregs all providing similar findings. Using a marker that differentiates between naïve and memory Tregs (CD45RA) they were also able to show that the most pronounced differences between

patients with coronary disease and controls are in the naïve Treg population. It remains to be clarified what the reduced naïve Treg frequency and function in coronary disease patients stands for but it could potentially represent an impaired ability to control autoimmunity.

As pointed out by the authors of the observation of reduced Tregs in coronary disease patients does not alone provide evidence for a functional role of these cells in the disease process. However, it is still well in line with the athero-protective effects of Tregs observed in experimental studies as well as with a previous prospective study demonstrating that subjects with low Treg levels are at increased risk for development of coronary events [8]. However, if we assume that Tregs indeed are athero-protective also in humans how would they function? Activation of Tregs requires binding of an antigen to its T cell receptor. Accordingly, Tregs need to be specific for self-antigens that are encountered in atherosclerotic plaques for example lipoproteins. We know that oxidized LDL in plaques is targeted by Th1 cells that fuel plaque inflammation upon recognition of their cognate antigens. If Tregs specific for LDL antigens are present and activated at the same location they will suppress the auto-reactive Th1 cells by consuming the IL-2 these require to function (figure). As discussed above there is also evidence of a population of T effector cells with reactivity against normal apolipoprotein B-100 and that these can promote the development of atherosclerosis. That such cells exist is not entirely unexpected

because the immune system is forced to allow some auto-reactivity not to compromise the immunological diversity required for an effective infectious defence. The control of such autoreactive T cells requires a presence of Tregs with similar antigen specificity. It is possible that a general reduction of Treg frequency and function, such as described for patients with coronary disease in the paper by Hasib and co-workers, could shift this delicate balance towards pathogenic autoimmunity. Another possibility could be that Tregs inhibit plaque inflammation by a so called "bystander effect". In this scenario Tregs become activated when recognizing their cognate antigens in atherosclerotic plaques and inhibit inflammatory activation of surrounding cells by releasing anti-inflammatory cytokines such as IL-10 and TGF-β (figure).

Several experimental studies have demonstrated athero-protective effects of immunization with plaque-related auto-antigens and activation of Tregs has been a common feature in these studies [9]. The present findings of Hasib and co-workers add support the possibility of translating such approaches into clinical therapies. On a final note it should be recognized that Lena Jonasson, who was first to describe the involvement of adaptive immunity in human atherosclerotic plaques, now almost 30 years later has pioneered in demonstrating the clinical importance of this observation.

Conflict of interest statement

Jan Nilsson is signed as co-inventor on patents describing immune-modulatory therapy for atherosclerosis.

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Figure legend

Stabilizing functions of regulatory T cells (Tregs) in atherosclerotic plaques.

Activated macrophages release pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 that fuels plaque inflammation. They also release matrix metalloproteinases (MMPs)

that contributes to plaque destabilization by degrading fibrous material. Th1 T cells that become activated when recognizing their cognate antigen (for example derived from oxidized LDL) on macrophage MHC class II molecules will release interferon (IFN)-γ which in turn enhances the secretion of pro-inflammatory cytokines and MMPs from the macrophage. Activation of Tregs in atherosclerotic lesions may reduce inflammation by suppressing auto-reactive Th1 T cells. This is achieved primarily by the removing the interleukin (IL-2) Th1 T cells need to proliferate from the extracellular space. They may also inhibit plaque inflammation by secreting anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β.