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Review

Emerging biomarkers and intervention targets for immune-modulation of atherosclerosis – A review of the experimental evidence

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ABSTRACT

The role of inflammation in atherosclerosis and plaque vulnerability is well recognized. However, it is only during recent years it has become evident that this inflammation is modulated by immune responses against plaque antigens such as oxidized LDL. Interestingly, both protective and pathogenic immune responses exist and experimental data from animal studies suggest that modulation of these immune responses represents a promising new target for treatment of cardiovascular disease. It has been proposed that during early stages of the disease, autoimmune responses against plaque antigens are controlled by regulatory T cells that inhibit the activity of auto-reactive Th1 effector T cells by release of anti-inflammatory cytokines such as IL-10 and TGF-β. As the disease progresses this control is gradually lost and immune responses towards plaque antigens switch towards activation of Th1 effector T cells and release of pro-inflammatory cytokines such as interferon-γ, TNF-α and IL-1β. Several novel immune-modulatory therapies that promote or mimic tolerogenic immune responses against plaque antigens have demonstrated athero-protective effects in experimental models and a first generation of such immune-modulatory therapies are now in early or about to enter into clinical testing. A challenge in the clinical development of these therapies is that our knowledge of the role of the immune system in atherosclerosis largely rests on data from animal models of the disease. It is therefore critical that more attention is given to the characterization and evaluation of immune biomarkers for cardiovascular risk.

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Current therapies for prevention of cardiovascular disease rest almost exclusively on risk factor intervention. This approach has proven very successful and experience from randomized clinical trials has shown that up to 40% of cardiovascular events can be prevented by optimal medical risk factor intervention [1]. However, in spite of these encouraging results the majority of treated patients still receive insufficient protection. It is likely that to further improve the efficacy of cardiovascular prevention new treatments directly targeting the disease process in the arterial wall need to be developed. Such treatments will need to specifically target atherosclerotic plaque inflammation. In this review we will discuss

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evidence suggesting that this can be achieved by modulating immune responses against plaque antigens as well as the need of developing validated biomarkers that can be used to measure such immune responses.

The concept of the immune system as an attractive target for future cardiovascular therapies is primarily based on experimental studies demonstrating that inhibition of inflammatory mediators and induction of specific immune responses can reduce atherosclerosis burden [2–5]. The proposed pleiotropic and anti-inflammatory effects of statins and the usefulness of the inflammatory marker high-sensitivity C-reactive protein (hsCRP) in risk prediction in humans reinforce this notion [6,7]. On the other hand, the failures of non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 specific inhibitors have taught us that general anti-inflammatory treatment may not be a viable option for the treatment of atherosclerosis and the targeting of more specific immune responses will be needed [8,9]. Therefore, CRP, predominantly secreted by the liver and adipose tissue in response to inflammatory stress, is a relatively crude marker for the evaluation of specific immune responses in the vascular wall. It has become increasingly clear that to truly understand the role of inflammation in atherosclerosis CRP will be insufficient and novel biomarkers assessing the complex role of the immune system in the disease need to be developed. There is today convincing evidence for presence of immune responses against plaque antigens in atherosclerosis and it has been proposed that disease progression occurs as a result of a loss of immune tolerance against these antigens in the plaque [10]. However, atherosclerosis is probably not an autoimmune disease in the classical sense but rather a state of local immune dysfunction resulting in an imbalance between naturally occurring autoimmunity and the regulatory immune cells that should control this autoimmunity. The circumstances that women, which generally are more prone to develop autoimmune diseases, suffer from cardiovascular disease at an older age than men also suggest that atherosclerosis is not a true autoimmune disease.

1. Atherosclerosis and the immune system

An impressive list, encompassing almost all aspects of the immune system, can be compiled when trying to summarize immune mechanisms that contribute to atherosclerosis development in mice (Fig. 1). Depletion of monocytes, the precursors of macrophage foam cells in plaques, can to a large extent abrogate atherosclerosis development in mice [11,12]. Interestingly, this effect is more prominent at early stages of the disease while depleting monocyte/macrophages from advanced lesions does not alter plaque size or composition [13]. Dendritic cells have been implicated in the formation of the earliest detectable plaques [14,15]. Neutrophils are found in increased numbers in hypercholesterolemic mice and neutrophil depletion effectively reduces atherosclerosis if neutrophil-depleting antibodies are administered during early atherosclerosis development [16,17]. A concept has been put forth where neutrophils pave the way for monocyte infiltration during early atherosclerosis development [18]. Platelets also assist in the recruitment of immune cells to the atherosclerotic plaque by facilitating interactions between endothelial cells and monocytes, neutrophils, dendritic cells and T cells [19]. T cell responses have generally been regarded as pro-atherogenic, although protective T cell responses also exist. In this context it is interesting to note that cardiovascular complications are more common in human immunodeficiency virus-infected individuals than in age-matched uninfected individuals and that antiretroviral therapy, which increases T cell counts, reduces the cardiovascular risk in treated subjects [20].

2. T cells modulate inflammation in atherosclerosis

Initial studies in severely immune-deficient mice provided inconclusive and partly contradictive findings regarding the role of the immune system in atherosclerosis [21–23]. It was later revealed that this was explained by the fact that both protective and atherogenic immune responses exist [24]. There is now convincing evidence that type 1 T helper (Th1) cells promote disease and deletion of the transcription factor T-bet, which is required for Th1 lineage commitment, reduces atherosclerosis [25,26]. The role of type 2 T helper (Th2) cells in atherosclerosis is unclear as several Th2 cytokines have been assigned protective roles, whereas deletion of IL-4, the proteotypic cytokine for Th2 cells, reduces atherosclerosis development in some studies [27–29]. The role of Th17 in atherosclerosis is also debated as conflicting reports exist [30–32]. In contrast to $CD4^+$ T helper cells, $CD8^+$ cytolytic T lymphocytes have not been extensively studied, even though $CD8^+$ T cells are abundant in atherosclerotic plaques and they have been shown to be activated in hypercholesterolemic *Apoe*$^{-/-}$ mice [33]. Activation of $CD8^+$ T cells has also been associated with increased plaque formation in these mice [34]. In apparent contrast with these observations, atherosclerosis protection achieved by immunization with apolipoprotein B (apoB) peptide has been suggested to involve $CD8^+$ T cells [35]. On the other hand, Tap1-deficiency that leads to severely diminished $CD8^+$ T cell populations does not alter atherosclerosis development in *Apoe*$^{-/-}$ mice [36]. Regulatory T cells expressing the transcription factor FoxP3, on the other hand, are clearly limiting atherosclerosis development [37]. The development and activation of natural killer T (NK T) cells, a subset that expresses surface markers characteristic of both natural killer cells and conventional T cells, depends on the interaction of their T cell receptor with lipids and glycolipids presented on CD1d, an MHC-class I-type molecule. Deletion of CD1d, which also eliminates NK T cells, reduces atherosclerosis development, whereas administration of the exogenous activator αGalCer augments atherosclerosis [38,39]. The role of different immune cells in atherosclerosis is summarized in Fig. 1.

3. Emerging therapies promoting regulatory T cell responses

The atherosclerosis quenching properties of regulatory T cells have attracted much attention in recent years and have spurred the development of therapies that inhibit atherosclerosis in mice by promoting regulatory T cells. Regulatory T cells prevent autoimmunity by controlling the activity of potentially auto-reactive T cells that have escaped deletion in the thymus. These natural regulatory T cells are characterized by expression of CD25 and the transcription factor FoxP3, which is considered the master regulator of the regulatory T cell transcription program. A wealth of data supports a protective role for regulatory T cells in atherosclerosis. Mice lacking the co-stimulatory molecules CD80/86, CD28 or ICOS have reduced numbers of regulatory T cells and consequently develop atherosclerosis more readily [37,40]. Furthermore, depletion of regulatory T cells with an anti-CD25 antibody or by immunizations targeting FoxP3 also significantly increases the formation of atherosclerotic plaques [37,41]. Regulatory T cells generated in the periphery are characterized by expression of IL-10 (Tr1 cells) or TGF-β (Th3 cells). Adoptive transfer of a clone of ovalbumin-specific Tr1 cells together with its cognate antigen inhibits plaque development in mice and inhibition of Th3 cells through deletion of the receptor for TGF-β on T cells enhances disease progression [42,43]. Thus, inhibition of atherosclerosis has been associated with induction of several types of regulatory T cells including natural regulatory T cells in response to anti-CD3 and anti-CD45RB treatment [44,45], Th3 cells through oral immunization with oxidized

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