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THE PRESENT AND FUTURE

STATE-OF-THE-ART REVIEW

Vaccine for Atherosclerosis

Prediman K. Shah, MD,* Kuang-Yuh Chyu, MD, PhD,* Paul C. Dimayuga, PhD,* Jan Nilsson, MD, PhD†

ABSTRACT

Atherosclerosis is an immune-mediated inflammatory disease of the arterial wall, with both the innate and adaptive immune systems responding to many endogenous and exogenous antigens. Both proatherogenic as well as atheroprotective roles have been identified for the immune system in atherosclerosis. Hence, it is conceivable that an immunomodulatory strategy via active immunization against many of these antigens could potentially alter the natural history of atherosclerosis. This review discusses: 1) the complex role of important components of the innate and adaptive immune systems in atherogenesis; 2) the nature of many antigens that have been tested successfully in vaccine formulations to reduce atherosclerosis in pre-clinical experimental models; and 3) the potential opportunities and challenges for clinical application of vaccination for atherosclerosis in the future. (J Am Coll Cardiol 2014;64:2779–91) © 2014 by the American College of Cardiology Foundation.

Substantial data from experimental and clinical investigation support the role of immune-mediated inflammatory mechanisms in atherogenesis. Cells of both the innate and adaptive immune systems, such as macrophages, dendritic cells (DCs), B and T lymphocytes, and mast cells, are ubiquitous in atherosclerotic plaques (1,2). Immunoinflammatory mediators such as pathogen- and danger-associated molecular pattern molecules, immunoglobulins, cytokines, chemokines, and complement proteins are all present to varying degrees in the atherosclerotic plaque (3,4). In atherosclerosis-prone areas of the murine aorta, especially in the adventitia, antigen-presenting cells are already present in greater numbers than in atheroresistant segments, even before hyperlipidemia or atherosclerotic lesions are introduced, suggesting that there is immunoinflammatory priming in these segments (5).

INNATE IMMUNITY AND ATHEROSCLEROSIS. Innate immunity reflects a nonspecific immediate response to pathogens and other danger signals. Cells of the innate immune system, such as DCs and macrophages, act as sentinels and first responders, sampling the host environment to detect molecular signatures of damage or danger, such as oxidatively-modified low-density lipoprotein (oxLDL). These molecular signatures of damage or danger, which are perceived by the host as molecular insults to the vascular wall, interact with toll-like receptors. Toll-like receptors act as pattern-recognition receptors, leading to the activation of genes involved in acute inflammation and the eventual release of inflammatory cytokines that characterize the acute innate immune response. Disruption of genes involved in this innate immune signaling pathway reduces atherosclerosis, plaque inflammation, and circulating inflammatory proteins in mice, independent of changes in circulating cholesterol levels (6).

Proatherogenic roles for innate immune cells are demonstrated by the deletion of genes involved in their differentiation and proliferation. Hypercholesterolemic mice deficient in the macrophage colony-stimulating factor gene, which is essential

From the *Oppenheimer Atherosclerosis Research Center, Division of Cardiology, Cedars-Sinai Heart Institute, Cedars-Sinai Medical Center, Los Angeles, California; and the †Department of Clinical Sciences, Lund University, Malmö University Hospital, Malmö, Sweden. Funding for this study was provided by the Eisner Foundation, The Heart Foundation, the Spielberg Cardiovascular Research Fund, and Cardiovax, Inc. Drs. Shah, Chyu, and Nilsson are coinventors of an apoB-100-based peptide vaccine; patent rights are assigned to Cedars-Sinai Medical Center. Dr. Shah has served as a scientific advisor (unpaid) to Cardiovax LLC. Drs. Chyu and Dimayuga contributed equally to this work. Anthony DeMaria, MD, served as the Guest Editor for this paper.
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ABBREVIATIONS AND ACRONYMS

DC = dendritic cell

HSP = heat shock protein

Ig = immunoglobulin

LDL = low-density lipoprotein

LDLR = low-density lipoprotein receptor

MHC = major histocompatibility class

NK = natural killer

oxLDL = oxidatively-modified low-density lipoprotein

T_{reg} = regulatory T

for macrophage survival and proliferation, have markedly reduced atherosclerosis despite severe hypercholesterolemia (7). Accumulating evidence also suggests diversity in the subtypes of mononuclear cells involved in innate immune responses. Studies in mice identified 2 subtypes of monocytes, 1 of which is considered proinflammatory, bearing high Ly6C expression as a marker (8). This inflammatory subset of high Ly6C monocytes was proposed to be precursors of proinflammatory M1 macrophages found in the plaques. Another phenotypic subset of macrophages is M2 macrophages, which, in a simplified phenotypic classification, are involved in resolution of plaque (9,10). Although this simplified view of monocyte-macrophages helps investigators to better understand the phenotypic heterogeneity of monocyte-macrophages in vitro, real, in vivo phenotypic changes are likely much more complex and not quite so discrete (9–11).

Natural killer (NK) cells are another innate immune cell type that contributes to atherosclerotic lesion formation. Low-density lipoprotein receptor-deficient ($LDLR^{-/-}$) mice repopulated with bone marrow cells from Ly49A transgenic mice deficient in functional NK cells developed smaller atherosclerotic lesions in the aortic root and arch compared with $LDLR^{-/-}$ mice receiving bone marrow cells from nontransgenic donors (12). Depletion of NK cells in apolipoprotein (apo) $E^{-/-}$ mice using anti-asialo-GM1 antibodies reduced atherosclerosis. Adoptive transfer of wild-type NK cells into $apoE^{-/-}Rag\text{-}2^{-/-}$ interleukin $2rg^{-/-}$ mice augmented lesion size, confirming the proatherogenic role of NK cells. The proatherogenic function of NK cells appears to be dependent on mediators such as perforin and granzyme B (13).

Mast cells also accumulate in atherosclerotic lesions, and hypercholesterolemic mice lacking mast cells have reduced inflammation and lesion formation (14–16). Interestingly, 2 of these studies reported lower circulating cholesterol levels in mast cell-deficient mice, suggesting a link between innate mast cell signaling and cholesterol homeostasis (15,16).

DCs bridge the innate immune system with the adaptive immune response and are the upstream component in the chain of immune cell activation. This unique position makes DCs an important target when considering potential strategies to modulate atherosclerosis. Resident intimal DCs rapidly ingest lipid in hypercholesterolemic conditions, whereas depleting DCs using CD11c-specific diphtheria toxin receptor (DTR) transgenic mice resulted in reduced early lesion formation in $LDLR^{-/-}$ mice (17), supporting a proatherogenic role for conventional DCs. This study confirmed a previous report using genetic deletion of CD11c in $apoE^{-/-}$ mice (18). Murine and human atherosclerotic lesions contain plasmacytoid dendritic cells (pDCs). Exposure to oxLDL enables pDCs to elicit antigen-specific T cell responses. Depleting pDCs using antimouse plasmacytoid dendritic cell antigen-1 antibody in $apoE^{-/-}$ mice reduced atherosclerosis in the aortic sinus and was associated with reduced plaque inflammation and global suppression of T cell activation (19). The atherosclerosis-enhancing role of pDCs was mediated by activation of immune responses by autoantigens through protein-deoxyribonucleic acid (DNA) complexes (20).

ADAPTIVE IMMUNITY AND ATHEROSCLEROSIS. Compared with the blunt, nonspecific nature of the innate immune system, the adaptive immune response is more specific and develops over time through stochastic rearrangement during immunoblast development, generating a wide variety of T and B cell receptors that recognize specific antigens. Innate immune cells, such as DCs and macrophages, present antigens in the context of the major histocompatibility complex for recognition by T cells. T cell activation occurs upon presentation of the antigen in the setting of an inflammatory state, resulting in clonal proliferation. $CD8^+$ T cell clonal proliferation involves increased cytokine production and cytotoxic function targeted against cells presenting the specific antigen. $CD4^+$ T cell activation also results in cytokine production, which, in turn, skews subsequent B cell activation to produce specific immunoglobulins.

Prior work with B cells suggested a protective role against atherosclerosis in hypercholesterolemic mice (21). A series of elegant studies by the Witztum group showed that natural antibodies of the immunoglobulin (Ig) M isotype are reactive with the phosphorylcholine head group present in oxidized low-density lipoprotein (LDL), apoptotic cells, and the cell wall of Pneumococcus. These natural antibodies of the IgM isotype attenuated atherosclerosis (22–24), supporting the role of molecular mimicry in atherogenesis. These IgM antibodies are produced by self-renewing B1 cells, lending support to the protective role of B cells in atherogenesis (25). In contrast to these studies, B cell depletion using anti-CD20 antibody reduced atherosclerosis (26), suggesting that a more intricate balance of B cell subtypes is likely involved. Studies targeting the B cell activating factor pathway in murine atherosclerosis support this concept (27,28). B cell activating factor deletion resulted in reduced B2 cells with a preserved B1 cell population, and was associated with reduced atherosclerosis. Thus, cumulative evidence suggests that there is cell