Promise of Immune Modulation to Inhibit Atherogenesis*

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It is now widely recognized that the evolving atherosclerotic lesion has nearly all of the characteristics of a chronic inflammatory disease, including the active participation of many arcs of immune function. That immune mechanisms play an important, if not dominant, role once atherosclerosis has been initiated is attested to by scores of examples in which manipulations of both adaptive and innate immune function have profoundly impacted atherogenesis in murine models, accelerating it or providing atheroprotective influences (1–5).

Role of Immune Function in Atherogenesis

Most cellular components of the immune response such as monocyte/macrophages, dendritic cells, T cells, NKT and NK cells, and mast cells are present in lesions. Neutrophils are curiously absent, whereas B cells are not usually found in the lesion itself but in the immediate adventitia and in draining lymph nodes. In addition, noncellular components of immunity are prominently found in established lesions, including immunoglobulins, in part bound to specific disease-related antigens, such as oxidized low-density lipoproteins (oxLDL) (6).

In general, T lymphocytes appear to mediate a net proatherogenic effect, although there is a growing body of evidence that special subsets of regulatory T cells may provide atheroprotective properties as well. B cells, however, appear to convey an atheroprotective function. For example, B-cell-deficient low-density lipoprotein receptor (LDLR)^−/−^ mice developed increased atherosclerosis (7), and splenectomy of apolipoprotein (apo) E^−/−^ mice, which decreased anti-oxLDL antibodies, also resulted in increased atherosclerosis, and this effect could be reversed by infusion of B cells from aged apoE^−/−^ mice (8). However, it is unclear which population of B cells conferred this protection and whether this was due solely to humoral immunity or, conceivably, to a newly emerging regulatory role of B cells (9). Furthermore, in contrast to most studies indicating an antiatherogenic role for T cells, passive transfer of T cells into the splenectomized mice also conferred atheroprotective properties (8). These data suggest the important principle that the impact of a given immune component may well be context dependent and caution must be used in study and interpretation of the complex roles of immune function in atherogenesis.

Oxidation of LDL Generates Immunodominant Epitopes in Lesions

There are many potentially important antigens within atherosclerotic lesions, including bacterial and viral antigens, heat shock proteins, and variously modified proteins. There is now a large body of evidence that "oxidation-specific" neo-epitopes derived from the oxLDL constitute one class of immunodominant antigens. Indeed, both adaptive and innate arcs of immunity are involved in an orchestrated response to the many oxidized lipids and oxidized lipid-apolipoprotein B adducts generated (2). Two widely used models have been studied: LDL oxidized by exposure to copper (Cu-oxLDL) and malondialdehyde (MDA) modification of LDL (MDA-LDL). Our laboratory reported the presence of autoantibodies to epitopes of Cu-oxLDL and MDA-LDL in rabbits and humans and demonstrated the presence of such autoantibodies in atherosclerotic lesions, in part as components of immune complexes with oxLDL. Change in titer of autoantibodies to Cu-oxLDL and MDA-LDL in murine models of atherosclerosis paralleled the progression or regression of lesion formation. We subsequently proposed that an immunization strategy could be developed to ameliorate the progression of atherosclerosis by showing that immunization of Watanabe heritable hyperlipidemic rabbits and LDLR^−/−^ mice with homologous MDA-LDL could inhibit atherogenesis (10,11). These observations have been confirmed and extended by others (12,13). Similarly, immunization with Cu-oxLDL is also atheroprotective (14,15). The mechanisms by which such immunizations produce atheroprotective immunity are poorly understood, and, in part, studies of these mechanisms are hindered by the fact that each of the immunogens used, Cu-oxLDL and even MDA-LDL, contains a complex and varied set of epitopes, which independently may affect different arcs of adaptive and innate immunity. Furthermore, although immunization of animals with modified...
homologous LDL is atheroprotective, a similar vaccine approach to test in humans would require the derivatization of autologous LDL, which would not be practical for large populations. To develop a generalized and safe vaccine requires the identification of the specific immunogenic oxidation-specific epitopes that provide the atheroprotective immunity. Ideally, such defined chemical moieties could then be formulated into one or more immunogenic vaccine approaches that could be widely administered.

**Oxidation-Specific Epitopes Are Recognized by Natural Antibodies**

Our original selection of MDA-LDL as an immunogen was based on the fact that it already represented a more restricted set of epitopes than Cu-oxLDL. However, reactions of MDA with ε-amino of lysine are complex and yield a variety of adducts, which will need to be defined in future studies.

In the case of Cu-oxLDL, an even greater array of chemically modified lipids and lipid-protein adducts are generated. Cholesterol-fed apoE-deficient mice have very high autoantibody titers to oxLDL, particular immunoglobulin M (IgM), which enabled the cloning of a large panel of IgM B-cell hybridomas from the spleens of these mice with specificity for Cu-oxLDL (16). Each of these, such as the prototypic EO6, bound to both the lipid and the apoB moiety of oxLDL, and specifically to the phosphocholine (PC) headgroup of oxidized phospholipids (oxPL), such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC), but not the PC of native phospholipids (17). Importantly, EO6 inhibited the uptake of oxLDL by macrophage scavenger receptors CD36 and SR-BI (18,19), as did POVPC linked to a peptide. Furthermore, similar to the binding to EO6, the PC moiety of oxPL was sufficient itself to mediate binding of oxLDL to CD36 (20). These data suggested that PC-specific IgM, such as EO6, could inhibit oxLDL uptake in vivo and in that way decrease macrophage uptake and foam cell formation.

Because all of these cloned autoantibodies were IgM, which are thought in large part to represent natural antibodies in uninfected mice, the genetic origins of the complementary determining region determining the antigen-binding site of these IgM were revealed to be genetically identical to a well characterized B-1 cell clone, T15, described over 30 years ago (21). T15 natural antibodies bind to PC covalently linked to the cell-wall polysaccharide (C-PS) of pathogens and provide optimal protection to mice from lethal infection with *Streptococcus pneumoniae* (22). EO6/T15 also bound to the PC of oxPL present on the plasma membrane of apoptotic cells, which are known to be undergoing oxidative stress, but did not bind to viable cells. These studies demonstrated molecular mimicry between the PC of oxPL present on oxLDL and apoptotic cells, on one hand, and the PC moiety present on pneumococcus and many other infections pathogens, on the other hand. This dual specificity for microbial and “self”-antigens has been described as a characteristic of natural antibodies (23).

The fact that the same T15 natural antibody that blocked the uptake of oxLDL by macrophages also bound the PC of common microbial pathogens suggested that the natural IgM antibodies may ameliorate atherosclerosis. To test this hypothesis, cholesterol-fed LDLR−/− mice were immunized with heat-inactivated PC-containing pneumococci, which induced a near monoclonal expansion of anti-oxLDL IgM (predominantly of the T15 clonotype) and significantly reduced atherosclerosis as measured at the aortic valve (24). Notably, there was essentially no induction of IgG titers to oxLDL. Plasma of these immunized mice had an enhanced ability to inhibit the uptake of oxLDL by macrophages. These data support the idea that anti-PC IgM antibodies of the T15 idiotype are atheroprotective in mice. Indeed, infusion of such an anti-PC IgM into apoE−/− mice inhibited accelerated vein graft atherosclerosis, although this failed to inhibit progression of lesions in native atherosclerosis (25).

**Immunization With PC-KLH Produces Atheroprotection**

In the current issue of the *Journal*, Caligiuri et al. (26) use another technique to enhance the titers of PC-specific antibodies in plasma of apoE-deficient mice. Mice immunized with PC covalently linked to the immunogenic heterologous protein keyhole limpet hemocyanin (KLH) had a 40% decrease in the extent of lesions measured at the aortic root compared with mice immunized with KLH alone or with phosphate-buffered saline (PBS). Importantly, these immunizations were conducted in the presence of CpG adjuvant, previously shown not to influence atherogenesis. Although the mechanisms by which the PC-KLH immunizations induced atheroprotection are unknown, experiments suggested that sera from PC-KLH–immunized mice decreased uptake of oxLDL compared with sera of PBS-immunized mice. This might contribute to decreased foam cell formation, consistent with earlier experiments with sera of pneumococcal immunization. However, in that earlier experiment (24), there was only an increase in IgM titer to oxLDL in plasma, reflecting the predominant TI-2 responses, mediated primarily through induction of natural B-1 cells. Because IgM do not bind to receptors on macrophages, they have the ability to bind to and block oxLDL uptake by macrophage scavenger receptors. In addition, IgM are increasingly recognized to have important immunomodulatory properties. In contrast, mice immunized with PC-KLH had increases in both IgG and IgM, consistent with major histocompatibility complex (MHC) class II T-cell–mediated responses. Theoretically, the presence of oxLDL/IgG immune complexes could lead to cross-linking of macrophage Fc receptors, activation of proinflammatory responses, or even enhanced uptake of
oxLDL via Fc receptors favoring foam cell formation. Indeed, global Fcy receptor deficiency confers atheroprotection in apoE<sup>−/−</sup> mice, indicating a proatherogenic role for the engagement of such receptors (27). Because different IgG isotypes are known to bind different Fcy receptors with different affinities and to have functionally different activities, a detailed analysis of the IgG isotypes induced by this immunization strategy may provide further insight.

Nevertheless, the net impact of increasing the anti-PC titers was atheroprotection in the setting tested. However, the long-range impact of this strategy needs to be tested, as well as the impact on atherogenesis at other sites in the aortic tree, because recent experience clearly suggests that interventions can not only be site-specific but can impact lesion formation differentially at different stages of lesion development (28). Furthermore, the consequences of the unusual localization of B cells within the lesions reported in the immunized mice of the present study needs to be understood.

**Application of Anti-PC Immunization Strategy in Humans**

The study by Caligiuri et al. (26) confirms and extends the observation that an immunization strategy that enhances anti-PC antibodies in mice is atheroprotective. Because the PC of KLH and that of the cell wall of *S. pneumoniae* shows immunologic identity to the PC of oxLDL, it would seem that such an immunization strategy would be applicable to humans. Indeed, it was demonstrated that the sera of patients recovering from pneumococcal pneumonia contained IgM antibodies to pneumococcal C-PS that significantly correlated with levels of anti-oxLDL IgM antibodies in the serum of KLH (24). Normal serum IgM in humans has been shown to bind PC (29,30) and sera of patients, with high titers of anti-PC IgM also bound to apoptotic cells (31). These data suggest that humans also have PC-specific IgM antibodies with microbial/oxLDL/apoptotic cell cross-reactivity. Notably, several recent studies suggest an inverse relationship between IgM titers to oxidation-specific epitopes and cardiovascular disease (32–34). Thus, these data support the idea that atheroprotective antibodies (and/or associated immune responses) do occur. However, there are also differences in the anti-PC responses in humans. Unlike in mice, IgG antibodies—in particular, IgG2—dominate the anti-PC response in humans (35), and patients with periodontal disease have increased anti-PC IgG2 titers exhibiting cross-reactivity between dental plaque bacteria and oxLDL (36).

Because children and high-risk adults are currently being immunized against pneumococcus, one might ask if this could be logically expected to provide atheroprotective immunity. In mice, the TI-2 immune response to PC occurs primarily through induction of natural B-1 cells and is robust. In contrast, in young children and in older adults responses to TI-2 antigens are blunted and responses to PC are insufficient to provide immunity. For this reason, current pneumococcal PS vaccines are made from multiple serotypes (usually 17 or, more recently, 23), which are covalently coupled to a variety of immunogenic carrier proteins, including diphtheria toxoid, tetanus toxoid, and meningococcal outer membrane complex. In turn, this leads to classic MHC-II responses to antigens other than PC. Further, such TI-2 antigens are thought to impair adaptive responses, and attempts have been made to reduce the presence of PC in such vaccines. Indeed, a recent brief report failed to find increases in oxLDL titers in a small cohort of children and adults immunized with the 23-valent vaccine (37). Nevertheless, in principle, if it were possible to develop optimal immunization strategies that led to immune responses directed against PC, as occurred in mice immunized with *S. pneumoniae* or PC-KLH, this might be of value in limiting the progression of lesion formation in humans. Such a strategy might be applicable to adults of young or middle age, when initiation and progression of atherosclerosis occurs.

The study by Caligiuri et al. (26) adds to a growing body of work demonstrating the feasibility of development of a vaccine approach to inhibiting the progression of atherosclerosis. Identification of atheroprotective antigens and development of novel vaccine approaches will be required for the implementation of such a strategy. Obviously a great deal of work will be needed to demonstrate that this approach can be effective and safe in humans. However, the possibility of retarding the development of this ubiquitous disease by a vaccine approach that is widely applicable is well worth the painstaking research that will be required to bring this idea to fruition.

**References**


