[Frontiers in Bioscience 12, 2171-2182, January 1, 2007]

Atherosclerosis, Antiphospholipid Syndrome, and Antiphospholipid Antibodies

Danielle Nicolo, Arun S. Varadhachary, Marc Monestier

Department of Microbiology & Immunology, Temple University School of Medicine, Philadelphia PA 19140, USA

TABLE OF CONTENTS

1. ABSTRACT

In antiphospholipid syndrome (APS) patients, some antiphospholipid antibodies (APA) are directed against negatively-charged phospholipids, while other APA are specific for phospholipid-proteins such as β2-glycoprotein I (β2GPI). Increased levels of oxidized low density lipoproteins (oxLDL) are present in atherosclerosis patients and these patients develop anti-oxLDL antibodies. Several studies have reported cross-reactivities between APA and anti-oxLDL antibodies, and some authors have suggested that most APA are specific for oxidized forms of phospholipids. In contrast, other studies report that APA react with both reduced and oxidized cardiolipin. In this context, we have re-examined the literature surrounding antibodies found in atherosclerosis and the APS. We have also included results from a panel of anti-phospholipid monoclonal antibodies from W/B mice, an APS model, which indicates that these antibodies do not display any preference for oxidized epitopes on lipid molecules.

2. INTRODUCTION

The APS is characterized by an increased risk of thrombosis, thrombocytopenia, and recurrent fetal loss. The main serological manifestation is the presence of high titers of APA. In APS patients, two main categories of APA have been identified: APA that are directed against negatively-charged phospholipids such as cardiolipin, and APA that are specific for phospholipid-binding plasma proteins, most of which recognize β2GPI (1-3). Several mouse models have been developed to study the pathogenic role of APA. The New Zealand White x BXSB F1 hybrids (W/B F1) mice have been identified as an animal model of APS. Beginning at 10 weeks of age, these mice develop a systemic autoimmune disease including autoantibodies to DNA, cardiolipin, and platelets, as well as progressive thrombocytopenia, lupus nephritis, and cardiovascular disease (4-6). Most W/B F1 males develop a degenerative vascular disease affecting mostly the coronary artery system, often resulting in myocardial infarctions (4). W/B

F1 mice produce IgG autoantibodies against cardiolipin that show increased binding in the presence of β2GPI (6; 7).

APA can also be detected in other autoimmune diseases, such as systemic lupus erythematosus, and in non-autoimmune conditions. APA are also remarkable for their propensity to react with other antigens. For instance, APA can cross-react with oxLDL, suggesting that lipid oxidation may be important for autoantibody recognition. Patients with atherosclerosis have increased levels of LDL oxidation, and spontaneously develop antibodies to oxidized LDL (8). Furthermore, recent studies have shown that β2GPI interacts with oxLDL (9). This interaction may form epitopes which resemble those formed from the interaction of β2GPI with cardiolipin, possibly providing the basis for this cross-recognition. Further, several groups have argued that APA exclusively recognize oxidized forms of phospholipids and LDL, but not the native or reduced forms of these molecules (10; 11). Many aspects about the role of APA remain undetermined. Are the same autoantibodies indeed present in apparently disparate diseases such as APS and atherosclerosis? Are phospholipids and phospholipid-binding proteins the true immunogens that elicit the production of APA? Do APA play a pathogenic role or are they simply serological markers of disease?

3. IMMUNOLOGY OF ATHEROSCLEROSIS

Atherosclerosis is a complex, multifactorial disease that leads to the narrowing and hardening of the arteries (12). Atherosclerosis can lead to a variety of clinical manifestations, including peripheral vascular disease, coronary artery disease, and stroke. Atherogenesis can be influenced by both genetic and environmental factors. Some of the well-characterized risk factors include smoking, hypertension, diabetes, repeated injury of vascular tissues, and elevated levels of low density lipoprotein-associated cholesterol. Yet these risk factors explain only a part of the epidemiological features of atherosclerosis, and in more recent studies, immunological mechanisms have been increasingly implicated in modulating atherogenesis. Atherosclerosis is now considered a chronic inflammatory process and a growing body of evidence points toward the involvement of both the humoral and cellular immune system (13).

3.1. Oxidative modification of LDL

OxLDL have been implicated as the preeminent atherogenic lipoprotein in humans. The oxidation process, which occurs as a continuum, modifies both the protein and lipid components of the LDL particle. This has made it quite difficult to define the exact structural changes that render the native LDL particle atherogenic. Lipid peroxidation can result in the formation of a variety of biologically active products, including peroxides, malondialdehyde (MDA), and 4-hydoxynonenal. Oxidation of the fatty acids or chemical modification of the free lysine amino groups of apoB alters the LDL particle to the extent that it is no longer recognized by the LDL receptor (14). *In vitro*, LDL can be oxidized using several techniques, with the copper sulfate method representing the most commonly utilized approach to generate oxLDL. Less severe modifications can be induced by ferrous oxidation, resulting in minimally modified (MM)-LDL (15; 16).

Although the physiologically relevant mechanisms of LDL oxidation have proven difficult to identify, a considerable amount of evidence shows that LDL are oxidatively-modified in vivo. OxLDL have been isolated from atherosclerotic lesions (17) and antioxidants such as butylated hydroxytoluene (BHT) can inhibit the development of atherosclerosis (18). To gain additional insight into the mechanisms of oxidative damage in the human artery wall, studies have focused on the detection of stable compounds in lesions that result from specific reaction pathways. Catalytically active metal ions, iron and copper in particular, have been isolated from tissue homogenates of atherosclerotic lesions suggesting that complexes of metal ions may stimulate LDL oxidation in vivo (19; 20). Lipoxygenases, which are cytosolic proteins that directly oxygenate polyunsaturated fatty acids, have also been suggested to play a role in the oxidative modification of LDL. In support of this, 15-lipoxygenase (15-LO) mRNA and protein, which is secreted by activated phagocytes, have been detected in human atherosclerotic lesions (21; 21; 22; 22). In addition, transfection of antisense 12-lipoxygenase, the murine equivalent to 15-LO, eliminated the 12-LO protein and inhibited LDL-induced monocyte chemotactic activity (23). Moreover, Cyrus and colleagues reported that disruption of the 12/15-LO gene diminishes atherosclerosis in apoE-/- mice, a mouse model of atherosclerosis (24). In addition, overexpression of 15-LO in vascular endothelium accelerates early atherosclerosis in atherosclerosis-prone LDL receptor-deficient mice (25).

Activated phagocytes secrete the catalytically active heme protein myeloperoxidase, which colocalizes with foamy macrophages in the cellular-rich regions of lesions (26), suggesting that myeloperoxidase may promote LDL oxidation in vivo. Nitric oxide, a relatively stable free radical that plays a role in a variety of biological events, may react with superoxide in vivo to form peroxynitrite, a reactive nitrogen species that promotes LDL oxidation (27). However, studies with hypercholesterolemic rabbits (28) and mice (29) have shown that nitric oxide itself can decrease fatty streak formation. This anti-atherogenic effect may be due to the ability of nitric oxide to inhibit platelet aggregation or LDL oxidation, or to promote vascular relaxation (30; 31). In addition, oxidative modifications of LDL can be mediated by a variety of cells that are involved in the development of atherosclerotic lesions. More specifically, exposure to cultured endothelial cells (32), to smooth muscle cells (SMC) (33), or to monocytes and neutrophils (34) can induce oxidative modifications of LDL in vitro. Although extensive modification of LDL usually occurs in the arterial intima, oxLDL have also been detected circulating in the bloodstream (35). These oxLDL in the plasma may accumulate in the arterial intima, yet it remains to be

elucidated exactly how these particles enter into and are retained by the intimal areas.

OxLDL bind with high affinity to several receptors, termed scavenger receptors, on the surface of macrophages. Macrophages are a predominant cell type in atherosclerotic lesions (36; 37), and oxLDL have been identified as a potential cause of macrophage recruitment and retention in lesions (38). Contrary to the regulation of the expression of the LDL receptor, this ingestion is not regulated by intracellular cholesterol levels, which allows the macrophages to become filled with lipids and to be converted into foam cells. SR-A, the prototypic scavenger receptor, has a long, collagen-like domain that may enable it to bind to oxLDL. In addition to SR-A, several other scavenger receptors including CD36, SR-B1, LOX-1, and SR-PSOX internalize oxLDL and may be important in atherosclerosis (39-42). The roles of both SR-A and CD36 in atherosclerosis have been studied more extensively using mouse models with genetic modifications. In knockout experiments, the deletion of either one of these receptors results in a significant reduction of the severity of experimental atherosclerosis in apoE-deficient mice, a mouse model of atherosclerosis (43; 44). These findings are consistent with the notion that the uptake of oxLDL by macrophages, leading to foam cell formation, is an important process in atherogenesis.

In addition to their ability to promote the formation of foam cells, oxLDL are also involved in the stimulation of chemotaxis (45) of monocytes and T lymphocytes, the differentiation (46) of monocytes, the proliferation of macrophages (47), and the activation of endothelial cell adhesiveness (48). OxLDL stimulate the expression of monocyte chemotactic protein-1 (MCP-1), macrophage colony stimulating factor, and granulocyte-macrophage colony stimulating factor by endothelial cells. They also induce increased monocyte adhesion to and transmigration through the endothelial layer (49-51) by upregulating cellular adhesion molecules, including intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and the selectins on the endothelial cell surface (52). Once they cross the endothelial layer, monocytes become trapped in the sub-endothelial space where they can interact with LDL that have been subjected to oxidative modifications.

3.2. Lesion development and composition

During atherogenesis, the lumen of the blood vessels becomes narrowed and eventually obstructed; lesions tend to form at the branch points of arterial blood vessels and progress through a variety of stages. Interestingly, oxLDL can be found in aortic samples from human fetuses even before signs of inflammation (53). The disease starts as a fatty streak lesion that is characterized predominantly by the presence of lipid-filled macrophages called foam cells. This aggregation of foam cells, macrophages, and T cells occurs within the sub-endothelial space and the intima, the innermost layer of the artery wall, of the blood vessel. Fatty streaks develop into intermediate lesions that are composed of layers of macrophages and SMC. Intermediate lesions progress into the more advanced, complex, occlusive lesions called fibrous plaques. Fibrous plaques consist of a central acellular area of lipid, derived from necrotic foam cells, covered by a fibrous cap, which contains SMC and collagen.

In the early stages of atherosclerotic lesion development, foam cells appear to be the most prominent cell type, yet as the lesion progresses, a necrotic core of lipid, oxidized lipid, and other cell debris accumulates. The formation of a fibrous cap that walls off the necrotic core from the endothelium results from inflammation-induced increases in extracellular matrix production (12). Over time, increased lesion size and local proliferation of SMC can cause the plaque to protrude into the lumen. Events, which may be linked to the local inflammatory response, can weaken the plaque resulting in plaque rupture, thrombosis, and the accompanying myocardial infarction or stroke (54). The final stage, called the complex lesion, shows evidence of thrombus formation with fibrin and platelet deposition.

Atherosclerotic lesions contain large numbers of immune cells, particularly macrophages and T cells. Recent evidence has implicated macrophages in the evolution of advanced lesions, which are characterized by intimal proliferation of SMC, matrix deposition, and lipid-rich necrotic cores. Macrophages secrete growth factors and cytokines that can facilitate lesion progression by promoting vascular cell migration and by creating an environment that promotes LDL modification (55). In addition, growth factors secreted by activated SMC, macrophages, and endothelial cells may stimulate SMC proliferation, another of the major factors responsible for the development of atherosclerotic lesions (56).

3.3. Immune Mechanisms in Atherosclerosis

Although it is well established that inflammation and the immune system play essential roles in atherogenesis, the exact mechanisms of pathogenesis remain to be elucidated. The development of atherosclerosis seems to involve both innate and adaptive immune mechanisms. In addition, recent evidence has demonstrated that both cell-mediated and humoral responses may modulate the development of atherosclerosis. Importantly, many recent advances in mouse models of atherosclerosis have enabled more detailed studies of the individual factors that may affect atherogenesis.

In an effort to more clearly define the immune mechanisms involved in the development of atherosclerosis, many studies have investigated the effects of both Th1 and Th2 responses on the process of atherogenesis. Th1 cells are associated with a pro-inflammatory, cell-mediated immune response because they result in the activation of T cells and macrophages. These T cells mainly produce the cytokines IL-2 and IFN-γ. IL-12, which is a growth factor for T cells produced mainly by activated monocytes, selectively biases T cells toward a Th1 response. In contrast, Th2 cells, which primarily produce the cytokines IL-4 and IL-5, are T cells that are involved in the modulation of the humoral immune response and serve to limit the Th1 response.

Investigations using gene knockout animal models support a role for pro-inflammatory responses in the progression of atherosclerosis. In general, a deficiency of the pro-inflammatory soluble factors that increase macrophage infiltration, adherence, and activation results in decreased development of atherosclerosis. ApoE knockout mice that are deficient in IFN-γ receptor show reduced lesion formation despite having elevated plasma cholesterol levels (57). MCP-1 is highly expressed in the macrophage-rich area of atherosclerotic lesions in both human and animal models (58). Furthermore, apoE-deficient mice with a genetic disruption in the gene for macrophage colony-stimulating factor (M-CSF) or MCP-1, both of which play key roles in proliferation of monocyte/macrophage cells, develop fewer atherosclerotic lesions (59; 60) than standard apoE-deficient mice. In support of these findings, the local over-expression of MCP-1 in the vessel wall of rabbit carotid arteries induced the infiltration of macrophages and the formation of atherosclerotic lesions (61). Additionally, MM-LDL can cause increased production of MCP-1 and M-CSF, which can lead to monocyte recruitment, proliferation, and differentiation (49; 60). Other pro-inflammatory cytokines have been detected in atherosclerotic lesions, including tumor necrosis factor (TNF)-α, IL-1β, and IFN-γ (62), yet their exact roles in the regulation of atherogenesis remains unclear.

Additional studies have examined the role of the humoral immune system on the development of atherosclerosis. In contrast to the effects of pro-inflammatory cytokines, IL-10 may play a counter-regulatory role in deactivating macrophages and T cells and, thus, in limiting atherogenesis. In situ hybridization studies have identified IL-10 in early and advanced human atherosclerotic lesions (63). In studies with IL-10 transgenic mice, IL-10 significantly reduced atherosclerosis in mice without affecting total cholesterol levels (64).

A few studies have focused on delineating the cross-regulatory roles of IL-10 and IL-12 in the development of atherosclerosis (65; 66). Uyemura and colleagues found that oxLDL, but not MM-LDL, could induce *in vitro* the release of the pro-inflammatory Th1 cytokine, IL-12 p70, from monocytes (65). IL-12 and IFN-γ were also detected in the atherosclerotic lesions. In addition, Uyemura and colleagues also indicated a role for IL-10 in limiting the local immune response in atherosclerosis. Their data show that IL-10 is present in some of the atherosclerotic lesions and that recombinant IL-10 could inhibit LDL-induced IL-12 production. Uyemura and colleagues concluded that the balance between IL-12 and IL-10 production contributes to the level of immune-mediated tissue injury, and thus, the severity of atherosclerosis (65). Additionally, Varadhachary et al. demonstrated that native LDL, upon binding to the LDL receptor, induced macrophages to produce the anti-inflammatory, Th2 cytokine IL-10; however, oxLDL induced IL-12 production from macrophages, which biases T cells toward a pro-inflammatory Th1 pathway (66).

T cells also participate in both early and advanced atherosclerotic lesion development, yet their exact role in atherogenesis remains to be elucidated. Most of the T cells in human plaques are CD4+ TCRαβ+ cells that express CD45RO and VLA-1, suggesting that these represent memory T cells in a state of chronic activation (67; 68). The presence of activated T cells in plaques has led to the speculation that these cells may affect lesion development by the local secretion of Th1 cytokines, including IFN-γ, IL-2, and TNF-α and -β (69). These Th1 cytokines can result in macrophage activation, vascular activation, and inflammation. Several conflicting studies provide evidence for and against the relevance of T lymphocytes in the development of atherosclerosis. In support of a pro-atherogenic role for T cells, studies in atherosclerosis-prone mice lacking IFN-γ (70), the IFN-γ receptor (57), or the p55 component of the TNF receptor (71) had decreased atherosclerosis compared to control mice. Contrary to these findings, cyclosporin treatment of hypercholesterolemic rabbits accelerated atherosclerosis suggesting a protective role for T lymphocytes (72). In addition, MHC class I-deficient C57BL/6 mice, which lack cytotoxic T cells, developed increased arterial lesions on a high-fat diet as compared to control mice (73).

4. ANTIBODIES IN THE ANTIPHOSPHOLIPID SYNDROME AND IN ATHEROSCLEROSIS

APA represent a heterogeneous group of circulating autoantibodies that can be detected in the sera of patients with autoimmune and infectious diseases, APS, and healthy individuals. One of the main issues under investigation with respect to APS is whether the APA are themselves pathogenic, rather than simply an epiphenomenon of the syndrome. The difficulty in understanding the role of APA is further compounded by the fact that APS patients often simultaneously express sets of APA with different binding specificities and that a combination of these specificities may be required for pathogenic outcomes. Thus, the exact relationship between APA and the APS remains controversial. APA have also been detected in the normal population, unassociated with autoimmune disease, but frequently following infections (74; 75). A few years ago, several studies started to suggest that APA associated with autoimmune diseases may recognize lipoproteins (76; 77)or phospholipid-protein complexes (76-78), while the antibodies detected in non-autoimmune individuals limit their specificity to phospholipids only. It is unclear whether membrane phospholipids play a role in eliciting APA. Anionic phospholipids are usually absent on the surface of most normal cells and the lack of reactivity of APA with intact cells suggest that disruption of the cell membrane, redistribution or modification of membrane phospholipids may be involved in triggering the production of APA. Indeed, some APA react with cells that have undergone alterations in the normal asymmetric distribution of membrane phospholipids and have exposed anionic phospholipids on their cell surface, such as activated platelets (79) and apoptotic cells (80). In considering the heterogeneity of APA, it may be likely that different cell types may bind to these antibodies and each may interact

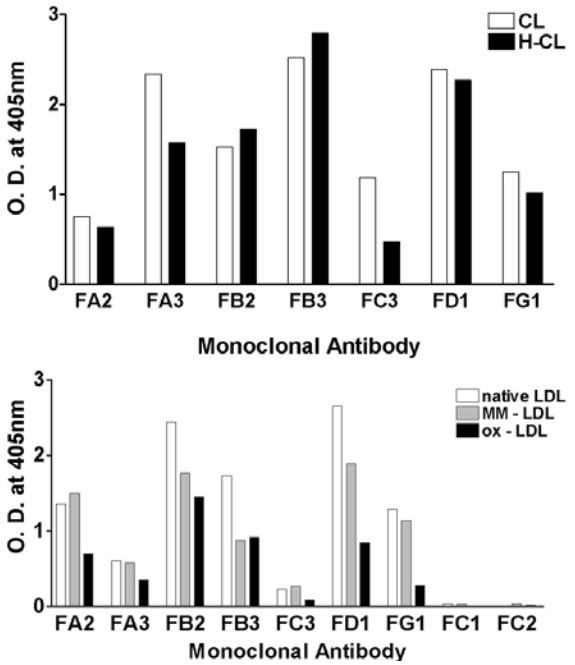

Figure 1. A (Top). Anti-phospholipid monoclonal antibodies bind similarly to cardiolipin (CL) and hydrogenated CL (H-CL). The ELISA was performed with 20ug/ml CL and H-CL and 20 ug/ml IgG antiphospholipid monoclonal antibody. B (Bottom). Antiphospholipid monoclonal antibodies crossreact with LDL particles, but do not preferential bind oxidized epitopes of LDL particles. The ELISA was performed with 20µg/ml LDL, minimally modified LDL (MM-LDL), or oxidized LDL (ox-LDL). The monoclonal antibodies from NZW x BXSB/F_1 mice were used at 20 µg/ml. FC1 and FC2 are anti-β2GPI monoclonal antibodies.

with an APA population of a different specificity. Thus, APA probably consist of a mixture of antibodies that are broadly cross-reactive and of antibodies that are more limited in their specificity.

Elevated titers of APA may contribute significantly to cardiovascular disease in patients with systemic lupus erythematosus (81-84) and APS (85; 86). More specifically, accelerated atherosclerosis, myocardial infarction, and cerebral thrombosis occur with increased frequency in patients with systemic lupus erythematosus (85; 87). Anti-cardiolipin antibodies may contribute to occlusive thrombosis and cardiovascular disease by inducing platelet activation and causing abnormal platelet aggregation (88; 89). OxLDL can promote coagulation in the endothelial environment by inducing macrophages to release of tissue factor, a major pro-coagulant and trigger to thrombosis associated with plaque formation. OxLDL also stimulate coagulation by modulating the tissue factor pathway inhibitor (90) and by reducing thrombomodulin transcription (91). OxLDL can also stimulate platelet adhesion and aggregation by decreasing endothelial production of nitric oxide (92).

In contrast, some recent studies have suggested that certain APA may be protective against the formation of atherosclerotic plaques. In several animal models of atherosclerosis, immunization with LDL can protect against the progression of the disease. In hyper-cholesterolemic rabbits, the immune response against administered LDL reduced the extent of plaque formation (93). In atherosclerosis-prone LDL receptor-deficient or apoE-deficient mice, immunization with malondialdehyde-modified LDL (MDA-LDL) decreased disease development (94-96). MDA results from *in vivo* lipid peroxidation and antibodies against this modified form of LDL can be detected in mice and humans (97). In agreement with these studies, IgG antibody levels against MDA-LDL and oxidized cardiolipin inversely correlate with plaque formation in immunized apoE-/- mice (98). It has also been reported that some IgM antibodies from atherosclerosis-prone mice cross-react with phosphorylcholine, a component of certain bacterial cell-walls which is exposed on oxLDL, but not native LDL. Immunization with *S. pneumoniae* can elicit IgM and IgG3 antibodies that cross-react with oxLDL and decreases plaque formation in LDLR-/- mice (99). We have observed that the passive administration of FB1, a monoclonal IgG APA from a W/B F1 mouse decreased plaque formation by 33 % in LDL receptor-deficient mice when compared to animals receiving a control monoclonal antibody (100). Therefore, the humoral response to phospholipids and to LDL may represent a promising approach to the prevention or treatment of atherosclerosis. One of the major challenges in the field will be to identify the subsets of APA that possess such beneficial properties.

5. ANTIBODIES RECOGNIZING B2-GLYCOPROTEIN I

β2GPI, which has been implicated in the pathophysiology of APS and has been suggested to participate in the progression of atherosclerosis, may also be among the proteins that undergo oxidative modifications (101). APA, and more specifically anti-β2GPI antibodies, may recognize oxLDL in complex with β2GPI (102). Patients with APS produce antibodies to β2GPI, a plasma phospholipid-binding protein.

These antibodies usually react better in ELISA when β2GPI is associated with phospholipids (12). In a survey of a panel of monoclonal antibodies from W/B1 mice, we observed that FC1 and FC2, two anti-β2GPI IgG monoclonal antibodies, do not cross-react with LDL, irrespective of its oxidation state (Figure 1). Our data are in contrast with those of Mizutani et al.(103) who reported that two W/B F1 monoclonal antibodies to β2-GPI cross-reacted with oxLDL, but not with native LDL. These authors suggested that this cross-reactivity was due to the fact that oxLDL contains a single lipid-associated protein, apoB, that may be structurally similar to the CL-β2GPI complex (103). This hypothesis seems unlikely. β2GPI and apoB are not related proteins and are therefore unlikely to express common epitopes. APS antibodies react better with β2GPI adsorbed to CL-coated plates than to β2GPI

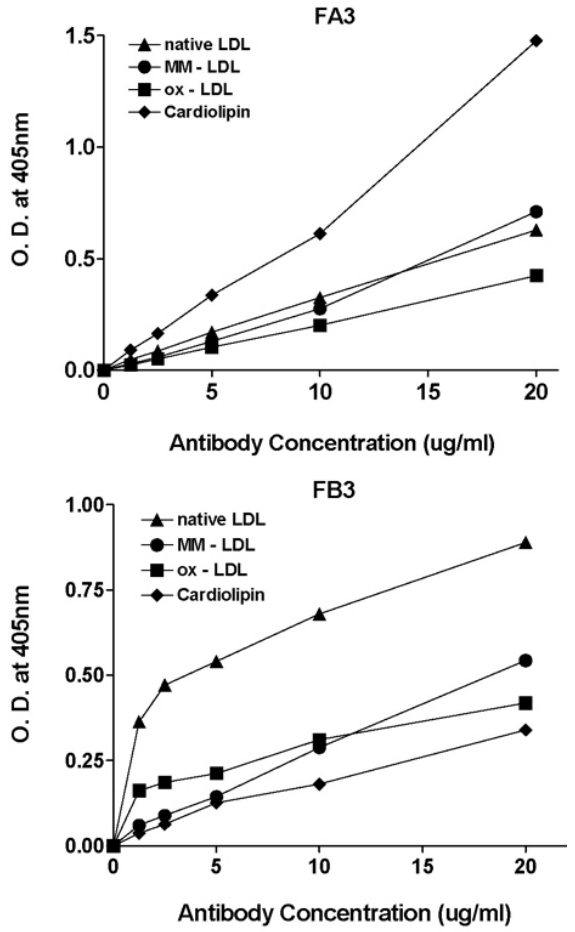

Figure 2. Examples of dose effect binding of antiphospholipid antibodies to cardiolipin and low density lipoproteins at various stages of oxidation. A (Top), The ELISA was performed using 20μg/ml of the antigen and varying amounts of the antiphospholipid monoclonal antibody FA3. B (Bottom), The ELISA was performed using 20μg/ml of the antigen and varying amounts of the antiphospholipid monoclonal antibody FB3.

alone because the binding of β2GPI to cardiolipin increases epitope density and not because of the formation of neoepitopes (104; 105). Indeed, most anti-β2GPI antibodies recognize domain I whereas domain V of β2GPI interacts with cardiolipin (106). It is therefore unlikely that the CL-binding region of β2GPI is directly involved in antibody recognition. β2GPI can bind oxidized plasma lipoproteins, including oxLDL, but does not react with native LDL (9). Since β2GPI is a very abundant plasma protein, a small amount of contaminating β2GPI in antibody or oxLDL preparations may be responsible for the reported cross-reactivities.

Studies from the Koike group have shown that β2-GPI can directly interact with oxidized plasma lipoproteins, including oxLDL, but does not bind native LDL (102; 107). These authors isolated an oxLDL-derived ligand (oxLig-1) specific for β2GPI (9). This ligand was identified as 7-ketocholesteryl-9-carboxynonanoate, one of the oxidation products of cholesteryl linoleate which is itself a predominant polyunsaturated fatty acid in LDL. The authors suggest that the interaction between native LDL and β2-GPI may not expose epitopes recognized by anti-β2GPI autoantibodies. In addition, they also isolated in APS patients with episodes of arterial thrombosis autoantibodies which recognized a complex of β2GPI and synthesized oxLig-1. This finding suggests that these complexes may serve as atherogenic autoantigens. Indeed, Lopez et al. demonstrated the presence of IgG antibodies to oxLDL/β2-GPI complexes in systemic lupus erythematosus patients with secondary APS and have shown a strong association with arterial thrombosis (108). They showed that systemic lupus erythematosus patients, especially those with secondary APS, had significantly higher levels of IgG anti-oxLDL/ β2GPI antibodies (108).

6. ANTIBODIES RECOGNIZING OXIDIZED AND NATIVE EPITOPES

Whether APA can recognize both native and oxidized lipid determinants is still an unresolved issue. One of the difficulties in assessing some earlier studies is that phospholipids, such as cardiolipin, oxidize quickly and spontaneously and that some studies performed with non-modified antigens may in fact have been unwittingly conducted with oxidized phospholipids. The preferential reactivity of APA with oxidized cardiolipin may stem from the fact that cardiolipin is the only phospholipid in mitochondria that undergoes early oxidation in mitochondria (16). It is becoming increasingly apparent that systemic autoimmunity is often associated with defects in the clearance of apoptotic material, which may lead to prolonged exposure to oxidized cardiolipin and thereby increase its immunogenicity.

Several authors have reported that anti-cardiolipin antibodies can cross-react with oxLDL (11; 103; 109; 110). This cross-reactivity may be related in part to the finding that cardiolipin is a component of LDL (111). Furthermore, the results of these studies suggest that APA bind to epitopes of oxidized phospholipids and lipoproteins, but not to the native, unmodified forms of these molecules (103; 112; 113). In addition, Boyd and colleagues showed that their panel of monoclonal anti-oxLDL antibodies reacted with oxLDL, but did not react with native LDL (114). These groups speculated that oxidation of these phospholipids and lipoproteins could result in the generation of modified phospholipid-protein or phospholipid-lipid adducts that are immunogenic. Because oxidation occurs on a continuum, a variety of oxidatively-modified phospholipids are generated when LDL undergoes even miminal amounts of oxidation. Some of these oxidatively-modified species of LDL have been detected in vivo, including oxidized LDL, MDA-LDL, and MM-LDL (17; 115; 116).

We screened a panel of monoclonal APA from W/B1 mice for binding to various forms of cardiolipin and low density lipoprotein (7). In contrast to previous studies, our APA bound similarly to both reduced and oxidized cardiolipin (Figure 1A). The reduced form of cardiolipin is

hydrogenated at all of the double bonds in its fatty acid chains, making this molecule extremely stable and resistant to oxidation. In screening our APA against native, MM-, and ox-LDL, most of the antibodies reacted better with native and MM-LDL than with oxLDL (Figure 1B). Figure 1 shows that the level of binding to LDL is not necessarily proportional to CL reactivity. For instance, FA3 binds CL better that FA2, but FA2 binds LDL better than FA3. As examples, two different dose-effect patterns are displayed in Figures 2A and 2B. FB3 prefers native LDL to both oxidized derivatives (Figure 2B). Conversely, FA3 reacts similarly with all three forms of LDL (Figure 2A). We also tested FC1 and FC2, two W/B monoclonal antibodies specific for β2GPI that do not bind CL (7). As mentioned above, neither reacts with LDL in its various forms (Figure 1B). Because oxidation is a continuous process, oxidation-specific epitopes of LDL are continuously created and destroyed. Our antibodies demonstrate higher levels of binding to the native and less oxidized species of LDL than to the fully oxidized LDL, suggesting that perhaps the antigenic epitopes that these antibodies originally recognized, in the native form of the molecule, are being destroyed or altered by the oxidation process.

Using IgM monoclonal antibodies isolated from apoE-deficient mice, Hörkkö et al. have suggested that most APA react specifically with oxidation-derived epitopes (112). They have reported that monoclonal antibodies to oxLDL derived from atherosclerosis-prone mice reacted with spontaneously oxidized cardiolipin, but not with reduced, hydrogenated cardiolipin. The difference between our antibody binding results and those previously published may be due to differences in the origin of the autoantibodies. These earlier studies were performed with IgM monoclonal generated from the apoE-/- mouse model of atherosclerosis whereas we examined a panel of IgG monoclonal antibodies from W/B F1 mice (an APS model). Likewise, the consensus that APA recognize oxidized epitopes in human disease is not universal. Schlame et al. have observed that oxidation of cardiolipin did not enhance its antigenicity. They reported that both monoclonal APA and APA from patients with APS react with both native and oxidized cardiolipin (117). Aho et al. have found that antibodies to negatively charged PL and antibodies to oxLDL are found in systemic lupus erythematosus patients, but that these two populations are different since only APA are associated with the development of thrombosis (118).

In addition to differences in autoantibody populations, it is also possible that variations in the assays may have caused the differences in the reactivities that we observed with our antibodies, as compared with those of the other groups. The numerous reports in the literature concerning variations in results due to the lack of standardized reagents and tests used in the characterization of APA support this explanation (119-121). Yet, the differences in the reactivities of the APA reported by each group may not simply be due to differences in experimental design, but may actually further confirm the heterogeneous binding characteristics of this group of antibodies.

7. PERSPECTIVES

There are many unsolved issues concerning the role of APA in atherosclerosis. A critical question is represented by the true specificity of these antibodies. Antibodies to negatively charged phospholipids are routinely detected in ELISA. In these tests, the lipids are adsorbed by evaporation onto a plastic plate, but this antigenic substrate bears little resemblance with the true configuration of phospholipids in the plasma membrane. Therefore some antibodies that react with phospholipids by ELISA may in fact be directed against a cross-reactive antigen or they may be polyspecific in nature. Antibodies to phospholipid-binding proteins are probably more restricted in their specificities, but antigenic cross-reactivities have also been reported among β2GPI-binding antibodies (122). This diversity of specificities is probably responsible for the various biological properties associated with APA, i.e. they can be pathogenic during APS and some APA are protective in atherosclerosis. Therefore, one of the major challenges in the field will be to identify novel and unique antigenic specificities that are recognized by APA. A more accurate mapping of the determinants bound by these autoantibodies could allow the differentiation between pathogenic and protective APA. The identification of harmful epitopes in APS and beneficial ones in atherosclerosis may thus lead to new therapeutic approaches.

8. ACKNOWLEDGEMENT

This work was supported by grants from the NIH and AHA.

9. REFERENCES

1. Rand J H. Molecular pathogenesis of the antiphospholipid syndrome. *Circ Res* 90, 29-37 (2002)
2. Levine JS, D W Branch & J Rauch. The antiphospholipid syndrome. *N Engl J Med* 346, 752-763 (2002)
3. Nicolo D & M Monestier. Antiphospholipid antibodies and atherosclerosis. *Clin Immunol* 112, 183-189 (2004)
4. Hang L M, S Izui & F J Dixon. (NZW x BXSB)F1 hybrid. A model of acute lupus and coronary vascular disease with myocardial infarction. *J Exp Med* 154, 216-221 (1981)
5. Mizutani H, T Furubayashi, A Kuriu, H Take, Y Tomiyama, H Yoshida, Y Nakamura, M Inaba, Y Kurata & T Yonezawa. Analyses of thrombocytopenia in idiopathic thrombocytopenic purpura-prone mice by platelet transfer experiments between (NZW x BXSB)F1 and normal mice. *Blood* 75, 1809-1812 (1990)
6. Hashimoto Y, M Kawamura, K Ichikawa, T Suzuki, T Sumida, S Yoshida, E Matsuura, S Ikehara & T Koike. Anticardiolipin antibodies in NZW x BXSB F1 mice. A model of antiphospholipid syndrome. *J Immunol* 149, 1063-1068 (1992)
7. Monestier M, D A Kandiah, S Kouts, K E Novick, G L Ong, M Z Radic & S A Krilis. Monoclonal antibodies from NZW x BXSB F_1 mice to β_2-glycoprotein I and cardiolipin.

Species specificity and charge-dependent binding. *J Immunol* 156, 2631-2641 (1996)
8. Horkko S, C J Binder, P X Shaw, M K Chang, G Silverman, W Palinski & J L Witztum. Immunological responses to oxidized LDL. *Free Radic Biol Med* 28, 1771-1779 (2000)
9. Kobayashi K, E Matsuura, Q Liu, J Furukawa, K Kaihara, J Inagaki, T Atsumi, N Sakairi, T Yasuda, D R Voelker & T Koike. A specific ligand for beta(2)-glycoprotein I mediates autoantibody-dependent uptake of oxidized low density lipoprotein by macrophages. *J Lipid Res* 42, 697-709 (2001)
10. Horkko S, E Miller, D W Branch, W Palinski & J L Witztum. The epitopes for some antiphospholipid antibodies are adducts of oxidized phospholipid and beta2 glycoprotein 1 (and other proteins). *Proc Natl Acad Sci USA* 94, 10356-10361 (1997)
11. Vaarala O, G Alfthan, M Jauhiainen, M Leirisalo-Repo, K Aho & T Palosuo. Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet* 341, 923-925 (1993)
12. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 340, 115-126 (1999)
13. Hansson G K. Immune mechanisms in atherosclerosis. *Arterioscler Thromb Vasc Biol* 21, 1876-1890 (2001)
14. Steinberg D. Modified forms of low-density lipoprotein and atherosclerosis. *J Intern Med* 233, 227-232 (1993)
15. Berliner J A, M C Territo, A Sevanian, S Ramin, J A Kim, B Bamshad, M Esterson & A M Fogelman. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest* 85, 1260-1266 (1990)
16. Itabe H, M Mori, Y Fujimoto, Y Higashi & T Takano. Minimally modified LDL is an oxidized LDL enriched with oxidized phosphatidylcholines. *J Biochem* 134, 459-465 (2003)
17. Palinski W, M E Rosenfeld, S Yla-Herttuala, G C Gurtner, S S Socher, S W Butler, S Parthasarathy, T E Carew, D Steinberg & J L Witztum. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci USA* 86, 1372-1376 (1989)
18. Bjorkhem I, A Henriksson-Freyschuss, O Breuer, U Diczfalusy, L Berglund & P Henriksson. The antioxidant butylated hydroxytoluene protects against atherosclerosis. *Arterioscler Thromb* 11, 15-22 (1991)
19. Swain J & J M Gutteridge. Prooxidant iron and copper, with ferroxidase and xanthine oxidase activities in human atherosclerotic material. *FEBS Letters* 368, 513-515 (1995)
20. Lamb DJ, M J Mitchinson & D S Leake. Transition metal ions within human atherosclerotic lesions can catalyse the oxidation of low density lipoprotein by macrophages. *FEBS Letters* 374, 12-16 (1995)
21. Yla-Herttuala S, H Sumuvuori, K Karkola, M Mottonen & T Nikkari. Atherosclerosis and biochemical composition of coronary arteries in Finnish men. Comparison of two populations with different incidences of coronary heart disease. *Atherosclerosis* 65, 109-115 (1987)
22. Yla-Herttuala S, M E Rosenfeld, S Parthasarathy, C K Glass, E Sigal, J L Witztum & D Steinberg. Colocalization of 15-lipoxygenase mRNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. *Proc Natl Acad Sci USA* 87, 6959-6963 (1990)
23. Navab M, S Y Hama, G M Anantharamaiah, K Hassan, G P Hough, A D Watson, S T Reddy, A Sevanian, G C Fonarow & A M Fogelman. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. *J Lipid Res* 41, 1495-1508 (2000)
24. Cyrus T, J L Witztum, D J Rader, R Tangirala, S Fazio, M F Linton & C D Funk. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J Clin Invest* 103, 1597-1604 (1999)
25. Harats D, A Shaish, J George, M Mulkins, H Kurihara, H Levkovitz & E Sigal. Overexpression of 15-lipoxygenase in vascular endothelium accelerates early atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 20, 2100-2105 (2000)
26. Daugherty A, J L Dunn, D L Rateri & J W Heinecke. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 94, 437-444 (1994)
27. Graham A, N Hogg, B Kalyanaraman, V O'Leary, V Darley-Usmar & S Moncada. Peroxynitrite modification of low-density lipoprotein leads to recognition by the macrophage scavenger receptor. *FEBS Letters* 330, 181-185 (1993)
28. Cooke J P & P S Tsao. Is NO an endogenous antiatherogenic molecule? *Arterioscler Thromb* 14, 653-655 (1994)
29. Aji W, S Ravalli, M Szabolcs, X C Jiang, R R Sciacca, R E Michler & P J Cannon. L-arginine prevents xanthoma development and inhibits atherosclerosis in LDL receptor knockout mice. *Circulation* 95, 430-437 (1997)
30. Moncada S, R M J Palmer & E A Higgs. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43, 109-142 (1991)
31. Jessup W, D Mohr, S P Gieseg, R T Dean & R Stocker. The participation of nitric oxide in cell free- and its restriction of macrophage-mediated oxidation of low-density lipoprotein. *Biochim Biophys Acta* 1180, 73-82 (1992)
32. Morel D W, P E DiCorleto & G M Chisolm. Endothelial and smooth muscle cells alter low density lipoprotein in vitro by free radical oxidation. *Arteriosclerosis* 4, 357-364 (1984)
33. Heinecke J W, L Baker, H Rosen & A Chait. Superoxide-mediated modification of low density lipoprotein by arterial smooth muscle cells. *J Clin Invest* 77, 757-761 (1986)
34. Cathcart M K, D W Morel & G M Chisolm. Monocytes and neutrophils oxidize low density lipoprotein making it cytotoxic. *J Leukocyte Biol* 38, 341-350 (1985)
35. Avogaro P, G B Bon & G Cazzolato. Presence of a modified low density lipoprotein in humans. *Arteriosclerosis* 8, 79-87 (1988)
36. Orekhov A N, E R Andreeva, I A Mikhailova & D Gordon. Cell proliferation in normal and atherosclerotic human aorta: proliferative splash in lipid-rich lesions. *Atherosclerosis* 139, 41-48 (1998)
37. Rekhter M D & D Gordon. Active proliferation of different cell types, including lymphocytes, in human atherosclerotic plaques. *Am J Pathol* 147, 668-677 (1995)

38. Quinn M T, S Parthasarathy, L G Fong & D Steinberg. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc Natl Acad Sci USA* 84, 2995-2998 (1987)
39. Silverstein R L & M Febbraio. CD36 and atherosclerosis. *Curr Opin Lipidol* 11, 483-491 (2000)
40. Acton S, A Rigotti, K T Landschulz, S Xu, H H Hobbs & M Krieger. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 271, 518-520 (1996)
41. Sawamura T, N Kume, T Aoyama, H Moriwaki, H Hoshikawa, Y Aiba, T Tanaka, S Miwa, Y Katsura, T Kita & T Masaki. An endothelial receptor for oxidized low-density lipoprotein. *Nature* 386, 73-77 (1997)
42. Shimaoka T, N Kume, M Minami, K Hayashida, H Kataoka, T Kita & S Yonehara. Molecular cloning of a novel scavenger receptor for oxidized low density lipoprotein, SR-PSOX, on macrophages. *J Biol Chem* 275, 40663-40666 (2000)
43. Suzuki H, Y Kurihara, M Takeya, N Kamada, M Kataoka, K Jishage, O Ueda, H Sakaguchi, T Higashi, T Suzuki, Y Takashima, Y Kawabe, O Cynshi, Y Wada, M Honda, H Kurihara, H Aburatani, T Doi, A Matsumoto, S Azuma, T Noda, Y Toyoda, H Itakura, Y Yazaki & T Kodama. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* 386, 292-296 (1997)
44. Febbraio M, E A Podrez, J D Smith, D P Hajjar, S L Hazen, H F Hoff, K Sharma & R L Silverstein. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *J Clin Invest* 105, 1049-1056 (2000)
45. Quinn M T, S Parthasarathy & D Steinberg. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc Natl Acad Sci USA* 85, 2805-2809 (1988)
46. Frostegard J, J Nilsson, A Haegerstrand, A Hamsten, H Wigzell & M Gidlund. Oxidized low density lipoprotein induces differentiation and adhesion of human monocytes and the monocytic cell line U937. *Proc Natl Acad Sci USA* 87, 904-908 (1990)
47. Matsumura T, M Sakai, S Kobori, T Biwa, T Takemura, H Matsuda, H Hakamata, S Horiuchi & M Shichiri. Two intracellular signaling pathways for activation of protein kinase C are involved in oxidized low-density lipoprotein-induced macrophage growth. *Arterioscler Thromb Vasc Biol* 17, 3013-3020 (1997)
48. Frostegard J, A Haegerstrand, M Gidlund & J Nilsson. Biologically modified LDL increases the adhesive properties of endothelial cells. *Atherosclerosis* 90, 119-126 (1991)
49. Rajavashisth T B, A Andalibi, M C Territo, J A Berliner, M Navab, A M Fogelman & A J Lusis. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature* 344, 254-257 (1990)
50. Navab M, S S Imes, S Y Hama, G P Hough, L A Ross, R W Bork, A J Valente, J A Berliner, D C Drinkwater & H Laks. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J Clin Invest* 88, 2039-2046 (1991)
51. Witztum J L & D Steinberg. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 88, 1785-1792 (1991)
52. Calara F, P Dimayuga, A Niemann, J Thyberg, U Diczfalusy, J L Witztum, W Palinski, P K Shah, B Cercek, J Nilsson & J Regnstrom. An animal model to study local oxidation of LDL and its biological effects in the arterial wall. *Arterioscler Thromb Vasc Biol* 18, 884-893 (1998)
53. Napoli C, F P D'Armiento, F P Mancini, A Postiglione, J L Witztum, G Palumbo & W Palinski. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 100, 2680-2690 (1997)
54. Libby P. Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis, and stabilization. *Am J Cardiol* 86, 3-8 (2000)
55. Libby P, Y J Geng, M Aikawa, U Schoenbeck, F Mach, S K Clinton, G K Sukhova & R T Lee. Macrophages and atherosclerotic plaque stability. *Curr Opin Lipidol* 7, 330-335 (1996)
56. Ares M P, B Kallin, P Eriksson & J Nilsson. Oxidized LDL induces transcription factor activator protein-1 but inhibits activation of nuclear factor-kappa B in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 15, 1584-1590 (1995)
57. Gupta S, A M Pablo, X C Jiang, N Wang, A R Tall & C Schindler. IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. *J Clin Invest* 99, 2752-2761 (1997)
58. Yla-Herttuala S, B A Lipton, M E Rosenfeld, T Sarkioja, T Yoshimura, E J Leonard, J L Witztum & D Steinberg. Expression of monocyte chemoattractant protein 1 in macrophage- rich areas of human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci USA* 88, 5252-5256 (1991)
59. Smith J D, E Trogan, M Ginsberg, C Grigaux, J Tian & M Miyata. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. *Proc Natl Acad Sci USA* 92, 8264-8268 (1995)
60. Qiao J H, J Tripathi, N K Mishra, Y Cai, S Tripathi, X P Wang, S Imes, M C Fishbein, S K Clinton, P Libby, A J Lusis & T B Rajavashisth. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. *Am J Pathol* 150, 1687-1699 (1997)
61. Namiki M, S Kawashima, T Yamashita, M Ozaki, T Hirase, T Ishida, N Inoue, K Hirata, A Matsukawa, R Morishita, Y Kaneda & M Yokoyama. Local overexpression of monocyte chemoattractant protein-1 at vessel wall induces infiltration of macrophages and formation of atherosclerotic lesion: synergism with hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 22, 115-120 (2002)
62. Libby P, G Sukhova, R T Lee & Z S Galis. Cytokines regulate vascular functions related to stability of the atherosclerotic plaque. *J Cardiovasc Pharmacol* 25 Suppl 2, S9-12 (1995)
63. Mallat Z, C Heymes, J Ohan, E Faggin, G Leseche & A Tedgui. Expression of interleukin-10 in advanced human

atherosclerotic plaques: relation to inducible nitric oxide synthase expression and cell death. *Arterioscler Thromb Vasc Biol* 19, 611-616 (1999)
64. Pinderski Oslund L J, C C Hedrick, T Olvera, A Hagenbaugh, M Territo, J A Berliner & A I Fyfe. Interleukin-10 blocks atherosclerotic events in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 19, 2847-2853 (1999)
65. Uyemura K, L L Demer, S C Castle, D Jullien, J A Berliner, M K Gately, R R Warrier, N Pham, A M Fogelman & R L Modlin. Cross-regulatory roles of interleukin (IL)-12 and IL-10 in atherosclerosis. *J Clin Invest* 97, 2130-2138 (1996)
66. Varadhachary A S, M Monestier & P Salgame. Reciprocal induction of IL-10 and IL-12 from macrophages by low density lipoproteins and its oxidized forms. *Cell Immunol* 213, 45-51 (2001)
67. Stemme S, L Rymo & G K Hansson. Polyclonal origin of T lymphocytes in human atherosclerotic plaques. *Lab Invest* 65, 654-660 (1991)
68. Stemme S, J Holm & G K Hansson. T lymphocytes in human atherosclerotic plaques are memory cells expressing CD45RO and the integrin VLA-1. *Arterioscler Thromb* 12, 206-211 (1992)
69. Hansson G K. Cell-mediated immunity in atherosclerosis. *Curr Opin Lipidol* 8, 301-311 (1997)
70. Nagano H, R N Mitchell, M K Taylor, S Hasegawa, N L Tilney & P Libby. Interferon-gamma deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. *J Clin Invest* 100, 550-557 (1997)
71. Schreyer S A, J J Peschon & R C LeBoeuf. Accelerated atherosclerosis in mice lacking tumor necrosis factor receptor p55. *J Biol Chem* 271, 26174-26178 (1996)
72. Roselaar S E, G Schonfeld & A Daugherty. Enhanced development of atherosclerosis in cholesterol-fed rabbits by suppression of cell-mediated immunity. *J Clin Invest* 96, 1389-1394 (1995)
73. Fyfe A I, J H Qiao & A J Lusis. Immune-deficient mice develop typical atherosclerotic fatty streaks when fed an atherogenic diet. *J Clin Invest* 94, 2516-2520 (1994)
74. McNeil H P, C N Chesterman & S A Krilis. Immunology and clinical importance of antiphospholipid antibodies. *Adv Immunol* 49, 193-280 (1991)
75. Cabiedes J, A R Cabral & D Alarcon-Segovia. Hidden anti-phospholipid antibodies in normal human sera circulate as immune complexes whose antigen can be removed by heat, acid, hypermolar buffers or phospholipase treatments. *Eur J Immunol* 28, 2108-2114 (1998)
76. Cabiedes J, A R Cabral AR & D Alarcon-Segovia. Clinical manifestations of the antiphospholipid syndrome in patients with systemic lupus erythematosus associate more strongly with anti-beta 2-glycoprotein-I than with antiphospholipid antibodies. *J Rheumatol* 22, 1899-1906 (1995)
77. Galli M, P Comfurius, C Maassen, H C Hemker, M H de Baets, P J van Breda-Vriesman, T Barbui, R F Zwaal & E M Bevers. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 335, 1544-1547 (1990)
78. McNeil H P, J E Hunt & S A Krilis. New aspects of anticardiolipin antibodies. *Clin Exp Rheumatol* 8, 525-527 (1990)
79. Shi W, B H Chong & C N Chesterman. Beta 2-glycoprotein I is a requirement for anticardiolipin antibodies binding to activated platelets: differences with lupus anticoagulants. *Blood* 81, 1255-1262 (1993)
80. Price B E, J Rauch, M A Shia, M T Walsh, W Lieberthal, H M Gilligan, T O'Laughlin, J S Koh & J S Levine. Anti-phospholipid autoantibodies bind to apoptotic, but not viable, thymocytes in a beta 2-glycoprotein I-dependent manner. *J Immunol* 157, 2201-2208 (1996)
81. Sturfelt G, O Nived, R Norberg, R Thorstensson & K Krook. Anticardiolipin antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum* 30, 382-388 (1987)
82. Boey M L, C B Colaco, A E Gharavi, K B Elkon, S Loizou & G R Hughes. Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. *Br Med J Clin Res Ed* 287, 1021-1023 (1983)
83. Harris E N, A E Gharavi, M L Boey, B M Patel, C G Mackworth-Young, S Loizou & G R Hughes. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 2, 1211-1214 (1983)
84. Frostegard J. SLE, atherosclerosis, and cardiovascular disease. *J Intern Med* 257, 485-495 (2005)
85. Roman M J, J E Salmon, R Sobel, M D Lockshin, L Sammaritano, J E Schwartz & R B Devereux. Prevalence and relation to risk factors of carotid atherosclerosis and left ventricular hypertrophy in systemic lupus erythematosus and antiphospholipid antibody syndrome. *Am J Cardiol* 87, 663-666 (2001)
86. Vaarala O. Antiphospholipid antibodies and atherosclerosis. *Lupus* 5, 442-447 (1996)
87. Shortell C K, K Ouriel, R M Green, J J Condemi & J A DeWeese. Vascular disease in the antiphospholipid syndrome: a comparison with the patient population with atherosclerosis. *J Vascul Surg* 15, 158-165 (1992)
88. Sherer Y & Y Shoenfeld. Antiphospholipid antibodies: are they pro-atherogenic or an epiphenomenon of atherosclerosis? *Immunobiology* 207, 13-16 (2003)
89. Nityanand S, C Bergmark, U de Faire, J Swedenborg, G Holm & A K Lefvert. Antibodies against endothelial cells and cardiolipin in young patients with peripheral atherosclerotic disease. *J Intern Med* 238, 437-443 (1995)
90. Petit L, P Lesnik, C Dachet, M Moreau & M J Chapman. Tissue factor pathway inhibitor is expressed by human monocyte-derived macrophages : relationship to tissue factor induction by cholesterol and oxidized LDL. *Arterioscler Thromb Vasc Biol* 19, 309-315 (1999)
91. Ishii H, K Kizaki, S Horie & M Kazama. Oxidized low density lipoprotein reduces thrombomodulin transcription in cultured human endothelial cells through degradation of the lipoprotein in lysosomes. *J Biol Chem* 271, 8458-8465 (1996)
92. Mertens A & P Holvoet. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J* 15, 2073-2084 (2001)
93. Ameli S, A Hultgardh-Nilsson, J Regnstrom, F Calara, J Yano, B Cercek, P K Shah & J Nilsson. Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 16, 1074-1079 (1996)

94. Palinski W, E Miller & J L Witztum. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci USA* 92, 821-825 (1995)
95. Freigang S, S Horkko, E Miller, J L Witztum & W Palinski. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes. *Arterioscler Thromb Vasc Biol* 18, 1972-1982 (1998)
96. George J, A Afek, B Gilburd, H Levkovitz, A Shaish, I Goldberg, Y Kopolovic, G Wick, Y Shoenfeld & D Harats. Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis* 138, 147-152 (1998)
97. Huang G S, Z P Wang, S C Wang, T J Sun, R Chu & S J Mao. Intracellular generation of MDA-LYS epitope in foam cells. *Life Sciences* 65, 285-296 (1999)
98. Zhou X, G Caligiuri, A Hamsten, A K Lefvert & G K Hansson. LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. *Arterioscler Thromb Vasc Biol* 21, 108-114 (2001)
99. Binder C J, S Horkko, A Dewan, M K Chang, E P Kieu, C S Goodyear, P X Shaw, W Palinski, J L Witztum and G J Silverman. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. *Nature Med* 9, 736-743 (2003)
100. Nicolo D, B I Goldman & M Monestier. Passive administration of antiphospholipid antibody reduces atherosclerosis in LDLR-/- mice. *Arthritis Rheum* 48, 2974-2978 (2003)
101. George J, Y Shoenfeld & D Harats. The involvement of beta2-glycoprotein I (beta2-GPI) in human and murine atherosclerosis. *J Autoimmunity* 13, 57-60 (1999)
102. Hasunuma Y, E Matsuura, Z Makita, T Katahira, S Nishi & T Koike. Involvement of beta 2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clin Exp Immunol* 107, 569-573 (1997)
103. Mizutani H, Y Kurata, S Kosugi, M Shiraga, H Kashiwagi, Y Tomiyama, Y Kanakura, R A Good & Y Matsuzawa. Monoclonal anticardiolipin autoantibodies established from the (New Zealand white x BXSB)F1 mouse model of antiphospholipid syndrome cross-react with oxidized low-density lipoprotein. *Arthritis Rheum* 38, 1382-1388 (1995)
104. Sheng Y, D A Kandiah & S A Krilis. Anti-beta 2-glycoprotein I autoantibodies from patients with the "antiphospholipid" syndrome bind to beta 2-glycoprotein I with low affinity: dimerization of beta 2-glycoprotein I induces a significant increase in anti-beta 2-glycoprotein I antibody affinity. *J Immunol* 161, 2038-2043 (1998)
105. Roubey R A, R A Eisenberg, M F Harper & J B Winfield. "Anticardiolipin" autoantibodies recognize beta 2-glycoprotein I in the absence of phospholipid. Importance of Ag density and bivalent binding. *J Immunol* 154, 954-960 (1995)
106. Reddel S W, Y X Wang, Y H Sheng & S A Krilis. Epitope studies with anti-beta 2-glycoprotein I antibodies from autoantibody and immunized sources. *J Autoimmunity* 15, 91-96 (2000)
107. Roubey R A. Systemic lupus erythematosus, hypercoagulable states, and antiphospholipid antibodies. *Arthritis Care Res* 12, 155-156 (1999)
108. Lopez L R, D F Simpson, B L Hurley & E Matsuura. OxLDL/β2GPI complexes and autoantibodies in patients with systemic lupus erythematosus, systemic sclerosis, and antiphospholipid syndrome. *Ann NY Acad Sci* 1051, 313-322 (2005)
109. Erkkila A T, O Narvanen, S Lehto, M I Uusitupa & S Yla-Herttuala. Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol* 20, 204-209 (2000)
110. Horkko S, T Olee, L Mo, D W Branch, V L J Woods, W Palinski, P P Chen & J L Witztum. Anticardiolipin antibodies from patients with the antiphospholipid antibody syndrome recognize epitopes in both beta(2)-glycoprotein 1 and oxidized low-density lipoprotein. *Circulation* 103, 941-946 (2001)
111. Deguchi H, J A Fernandez, T M Hackeng, C L Banka & J H Griffin. Cardiolipin is a normal component of human plasma lipoproteins. *Proc Natl Acad Sci USA* 97, 1743-1748 (2000)
112. Horkko S, E Miller, E Dudl, P Reaven, L K Curtiss, N J Zvaifler, R Terkeltaub, S S Pierangeli, D W Branch, W Palinski & J L Witztum. Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. Recognition of cardiolipin by monoclonal antibodies to epitopes of oxidized low density lipoprotein. *J Clin Invest* 98, 815-825 (1996)
113. Horkko S, D A Bird, E Miller, H Itabe, N Leitinger, G Subbanagounder, J A Berliner, P Friedman, E A Dennis, L K Curtiss, W Palinski & J L Witztum. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J Clin Invest* 103, 117-128 (1999)
114. Boyd H C, A M Gown, G Wolfbauer & A Chait. Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am J Pathol* 135, 815-825 (1989)
115. Hammer A, G Kager, G Dohr, H Rabl, I Ghassempur & G Jurgens. Generation, characterization, and histochemical application of monoclonal antibodies selectively recognizing oxidatively modified apoB-containing serum lipoproteins. *Arterioscler Thromb Vasc Biol* 15, 704-713 (1995)
116. Palinski W, V A Ord, A S Plump, J L Breslow, D Steinberg & J L Witztum. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde- lysine in serum. *Arterioscler Thromb* 14, 605-616 (1994)
117. Schlame M, I Haller, L R Sammaritano & T J Blanck. Effect of cardiolipin oxidation on solid-phase immunoassay for antiphospholipid antibodies. *Thromb Haemost* 86, 1475-1482 (2001)
118. Aho K, O Vaarala, L Tenkanen, H Julkunen, T Jouhikainen, G Alfthan & T Palosuo. Antibodies binding to anionic phospholipids but not to oxidized low-density

lipoprotein are associated with thrombosis in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 14, 499-506 (1996)
119. Harris E N. Antiphospholipid antibodies. *Br J Haematol* 74, 1-9 (1990)
120. Helbert M, S Bodger, J Cavenagh, D D'Cruz, J M Thomas & P MacCallum. Optimising testing for phospholipid antibodies. *J Clin Pathol* 54, 693-698 (2001)
121. Cao G, J L Goldstein & M S Brown. Complementation of mutation in acyl-CoA:cholesterol acyltransferase (ACAT) fails to restore sterol regulation in ACAT- defective sterol-resistant hamster cells. *J Biol Chem* 271, 14642-14648 (1996)
122. Cocca B A, S N Seal, P D'Agnillo, Y M Mueller, P D Katsikis, J Rauch, M Weigert & M Z Radic. Structural basis for autoantibody recognition of phosphatidylserine-beta 2 glycoprotein I and apoptotic cells. *Proc Natl Acad Sci USA* 98, 13826-13831 (2001)

Key Words: Antiphospholipid Antibodies, Phospholipid, B2-Glycoprotein I, Atherosclerosis, Low Density Lipoproteins, Review

Send correspondence to: Dr. Marc Monestier, Department of Microbiology & Immunology, Temple University School of Medicine, 3400 North Broad Street, Philadelphia, PA 19140, Tel: 215-707-1977, Fax: 215-707-7788, E-mail: marcm@temple.edu

http://www.bioscience.org/current/vol12.htm