β2-glycoprotein I in Human and Murine Atherosclerosis

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Atherosclerosis is a complex and indolent histopathological process considered to be the most common underlying process in cardiovascular morbidity and mortality. Previously, only traditional risk factors (such as smoking, hypertension, diabetes, etc.) were believed to enhance atherosclerosis development. However, the formation of atheroma is increasingly recognized as an inflammatory process in the arterial wall, including the accumulation of macrophages and activated T lymphocytes, and thus it is not surprising that autoimmune factors have been shown to play a role in the atherosclerosis process [1]. These include involvement of autoantigens and autoantibodies in atherogenesis. We summarize here the data on the association between β2-glycoprotein I, anti-β2GPI antibodies and human and murine atherosclerosis.

β2GPI in the antiphospholipid syndrome

Human β2GPI is a 50 kDa plasma glycoprotein that avidly binds negatively charged surfaces and substances, and acts as an anti-coagulant in *in vitro* assays [2]. Schwarzenbacher et al. [3] recently described a model of β2GPI structure containing 326 amino acid residues and 4 glycans. According to this model, β2GPI is formed from four complement control protein modules and a fifth C-terminal domain that constitute an elongated J-shaped molecule. This latter domain, which carries a distinct positive charge, is an excellent counterpart for interactions with negatively charged amphiphilic substances [3]. Autoantibodies directed towards β2GPI are among the serological markers of the antiphospholipid syndrome, an entity that combines clinical features such as thrombosis and pregnancy morbidity with the presence of specific autoantibodies [4]. Although previously considered only as a co-factor in APS, the autoantigen in APS is probably β2GPI since it undergoes structural alteration by binding to negatively charged phospholipids and is thus recognized by antiphospholipid antibodies.

The presence of anti-β2GPI antibodies in APS is associated with reproductive morbidity as well as thrombosis. Regarding thrombosis, there are several mechanisms of action suggesting a direct cause and effect relationship between the presence of anti-β2GPI antibodies and a pro-coagulant state. One example is the β2GPI-induced reversal of protein S functional modulation by its plasma inhibitor [5]. Since monoclonal anti-β2GPI antibody succeeded in inhibiting this effect, adequate levels of the anticoagulant protein S could not be maintained. Therefore, it is not surprising that β2GPI autoantibodies are associated with thrombosis. Patients having thrombosis associated with systemic lupus erythematosus, a condition that is occasionally associated with secondary APS, usually have anti-β2GPI antibodies and lupus anticoagulant (89%), and anticardiolipin antibodies (78%) [6]. However, of all SLE patients with anticardiolipin antibodies but without β2GPI antibodies or lupus anticoagulant activity, only 6% have thrombosis [6]. In another study of patients with SLE (some of them with secondary APS), the presence of IgG β2GPI antibodies was strongly associated with a history of arterial thrombosis [7]. Apart from the whole molecule of β2GPI, specific parts of it might also contribute to thrombosis. GDKV is a synthetic peptide spanning Gly274–Cys288 in the fifth domain of human β2GPI and represents the phospholipid binding site of β2GPI [8]. Following murine immunization with this peptide, the mice developed antiphospholipid and anti-β2GPI antibodies. Using a model of thrombosis in mice, these antibodies induced larger thrombi that persisted for a longer duration. The thrombogenic properties of these antibodies were mediated through activation of endothelial cells as the antibodies increased the surface expression of E-selectin, ICAM-1 and VCAM-1 on endothelial cells [8].

β2GP1 = β2-glycoprotein 1
APS = antiphospholipid syndrome

SLE = systemic lupus erythematosus
ICAM = intracellular adhesion molecule
VCAM = vascular cell adhesion molecule

β2GPI, anti-β2GPI antibodies and atherosclerosis

The presence of activated lymphocytes, macrophages and endothelial cells within atherosclerotic plaques suggests that the immune system is involved in atherogenesis and its progression. Accordingly, several autoantigens associated with atherosclerosis and their respective autoantibodies have been identified and are under extensive research. Examples include heat shock proteins, oxidized low density lipoprotein and β2GPI [summarized in ref. 9]. In addition to its pro-coagulant activity, as evident from its clinical association with thrombosis and from interference with the coagulation system, there is strong evidence to support a pro-atherogenic effect of anti-β2GPI antibody. The presence of β2GPI has been confirmed within human atherosclerotic lesions obtained from carotid endarterectomies [10]. β2GPI is abundantly expressed within the sub-endothelial regions and the intimal-medial border of human atherosclerotic plaques, and it co-localizes with CD4+ lymphocytes [10].

Anti-β2GPI antibodies are considered pro-atherogenic, whereas β2GPI probably has anti-atherogenic effects. β2GPI could inhibit *in vitro* uptake of oxidized LDL by murine macrophages; and conversely, binding of oxidized LDL to macrophages was significantly increased by simultaneous addition of anti-β2GPI antibodies [11]. That observation is important since oxidized LDL uptake by macrophages leads to foam cell formation. Furthermore, Mizutani et al. [12] demonstrated a cross-reaction between monoclonal β2GPI-dependent anticardiolipin antibodies and oxidized LDL [12]. Additionally, it has been shown that in patients with SLE, anti-endothelial antibody levels were increased and these antibodies had shared antigenic epitopes with β2GPI, oxidized LDL and lysophosphatidylcholine [13].

The best evidence, however, for the pro-atherogenic role of anti-β2GPI comes from animal models. The pro-atherogenic effect of anti-β2GPI was evaluated in two transgenic mouse models. The apo-E knockout mice developed spontaneous hypercholesterolemia and atherosclerotic lesions similar in nature to human plaques, while the LDL receptor-deficient mice developed atherosclerotic plaques only when fed a high fat diet. Immunization of either mouse strain with β2GPI resulted in pronounced cellular and humoral response to β2GPI [14,15]. The immunized mice developed high titers of anti-β2GPI antibodies, whereas the non-immunized mice or mice immunized against ovalbumin did not develop these antibodies. This increase in anti-β2GPI titer was concomitant with larger atherosclerotic lesions in the immunized mice, and these lesions contained abundant CD4+ cells [14,15]. In addition, lymphocytes obtained from β2GPI-immunized LDL receptor-deficient mice were transferred intraperitoneally into syngeneic mice. This resulted in the formation of larger fatty streaks in the recipients, compared with mice that received lymphocytes from control mice [16]. T cell depletion of lymphocytes failed to induce this effect. Hence, β2GPI-reactive T cells could promote atherogenesis.

Conclusions and future implications

β2GPI and anti-β2GPI are anti- and pro-atherogenic, respectively. However, given that β2GPI is prevalent in human atherosclerotic plaques, it is possible that circulating anti-β2GPI antibodies or cells that encounter their target antigen within pre-existing atherosclerotic lesions might contribute to the local inflammation and to atherosclerosis aggravation.

An additional aspect is immunomodulation, which might prevent or decrease existing atherosclerotic plaques. A novel therapeutic modality involving β2GPI has been successfully tested in an animal model. Using a phage display library, three different peptides that bind specifically to monoclonal anti-β2GPI antibodies were generated. The passive infusion of any of these three antibodies to BALB/c mice resulted in the induction of experimental APS [17]. However, the manifestations of experimental APS could be prevented by subsequent infusion of the corresponding specific peptides, which neutralize the functional activity of monoclonal anti-β2GPI *in vitro*. Moreover, exposure of endothelial cells to anti-β2GPI monoclonal antibodies and their corresponding peptides led to the inhibition of endothelial cell activation, as shown by decreased expression of adhesion molecules and monocyte adhesion [17]. These peptides might thus also have an anti-atherogenic effect.

In conclusion, the involvement of β2GPI and anti-β2GPI in atherogenesis is an example of the involvement of the immune system in atherosclerosis. It also provides an additional target for prevention and treatment of atherosclerosis.

LDL = low density lipoprotein

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