Review
The induction of experimental vascular diseases by immunization with pathogenic autoantibodies

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Introduction

Natural autoantibodies are part of the natural immunity, a component of the immune system functioning as a first line of defense, and capable of destroying foreign agents on initial contact (1, 2). However, natural autoantibodies, in certain circumstances, may be pathogenic (2, 3).

Previously, we (7-16) and others (4-6) have reported on the induction of experimental models of autoimmune conditions in naive mice actively immunized with pathogenic autoantibodies. This method is based on Jerne's theory (17, 18) that the idiotypic determinant of each autoantibody is complemented by those of another, creating an idiotypic network through which immunoglobulin expression might be controlled. This is manifested by the generation of anti-idiotypic antibodies of two functional subsets: those that recognize determinants in the V region and do not involve the combining site for the elicit antigen, and those that represent internal images of the elicit antigen.

Upon stimulation with the autoantibody carrying a specific idiotype (Ab1), naive mice develop an anti-autoantibody (anti-Id = Ab2), and after several months generate an anti-anti-autoantibody (anti-anti-Id = Ab3) that may have similar binding characteristics to Ab1 (15). In addition, the mice develop a respective overt autoimmune condition, typically one associated with the given inducing antibody (Ab1), e.g. SLE upon immunization with anti-DNA antibody (7,8), primary antiphospholipid syndrome (APS) when immunized with anti-cardiolipin (aCL) antibody (10, 19) or ANCA-associated vasculitis upon immunization with anti-neutrophil cytoplasmic antibody (12, 20) (Fig. 1).

In the current paper we present our own and other researchers' experience with animal models for several vascular diseases mediated by pathogenic autoantibodies, among them anti-neutrophil cytoplasmic antibodies, anti-glomerular basement membrane, anti-endothelial cells and anti-phospholipid antibodies. These animal models are induced by immunization with either an autoantigen, which leads to the generation of a pathogenic autoantibody, or by immunization with the pathogenic autoantibody itself, which is followed by the emergence of the idiotypic network cascade.

Animal models of ANCA-associated vasculitis

Studies of anti-neutrophil cytoplasmic antibodies (ANCA) have come to form an increasingly prominent portion of the literature on autoimmune diseases in the last 15 years. Since the first description of ANCA in patients with segmental necrotizing glomerulonephritis (21), ANCA have been demonstrated in a number of primary vasculitic syndromes (22, 23). In most cases of systemic vasculitis, the primary target antigens for ANCA are proteinase-3 (PR-3) and myeloperoxidase (MPO) (24). Both enzymes are present in the secretory granules of neutrophils and monocytes. It is now well established that anti-PR-3 is a sensitive marker of Wegener's granulomatosis (WG), whereas anti-MPO is predominantly found in microscopic polyangiitis and Churg-Strauss syndrome (22). Besides being a useful diagnostic tool, their strong association with primary vasculitic syndromes suggests an important role for ANCA in the pathophysiology of the associated disease. Indeed, in recent years several animal studies have been reported on ANCA and vasculitis using various experimental approaches (23, 25).

Anti-MPO associated glomerulonephritis

In 1993 Brouwer *et al.* reported a model

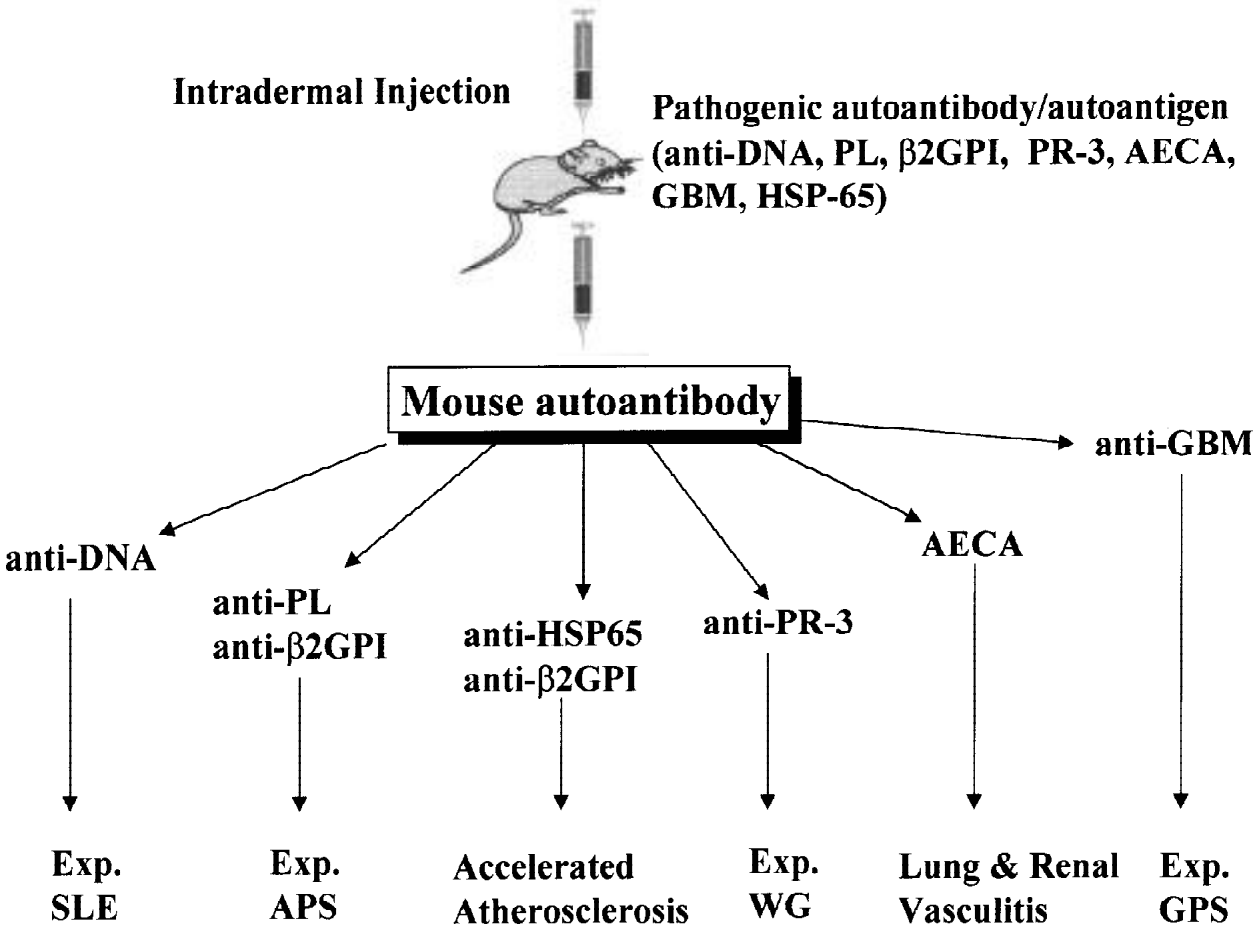

Fig. 1. Induction of autoimmune vascular diseased with pathogenic autoantibodies. Mice are immunized by an intradermal injection of a pathogenic autoantibody, in complete Freund's adjuvant (Ab1), followed by a boost injection after 3 weeks. The mice develop anti-idiotypic antibody (Ab2) and, later on, anti-anti-idiotypic antibody (Ab3) which may simulate Ab1 in its binding properties. This is followed by the emergence of the full-blown serological, immunohistochemical and clinical manifestations of the respective autoimmune disease.
AECA: anti-endothelial cell antibodies; APS: antiphospholipid syndrome; GBM: glomerular basement membrane; GPS: Goodpasture's syndrome; HSP: heat-shock proteins; PL: phospholipid, PR-3: Proteinase 3; SLE: systemic lupus erythematosus; WG: Wegener's granulomatosis.

for pauci-immune necrotizing crescentic glomerulonephritis in Brown Norway rats which closely resembles human anti-MPO associated pauci-immune glomerulonephritis (26). The disease was induced in MPO-immunized rats by unilateral kidney perfusion with a neutrophil lysosomal enzyme extract primarily consisting of MPO plus H_2O_2, the substrate of MPO, 5 weeks after immunization. MPO, IgG and C3 were present along the glomerular basement membrane at 24 hrs after perfusion, but were absent at 4 and 10 days. The immunized rats developed a proliferative glomerulonephritis characterized by intra- and extracapillary cell proliferation, periglomerular granulomatous inflammation, and the formation of giant cells (26). Granulomatous vasculitis of the small vessels was found at 10 days after perfusion.

Yang *et al.* (27), who repeated the study in spontaneously hypertensive and Brown Norway rats, reported that pathological lesions and deposits of IgG, C3, and MPO were continuously found in immunized rats perfused with MPO + H_2O_2, or MPO alone. The degree of histologic injury was proportional in intensity to the amount of IgG immune deposits. Their results imply that this rat model might be an example of immune complex-mediated rather than pauci-immune glomerulonephritis. Differences in the immunization procedure or the composition of neutrophil lysosomal extracts might have accounted for the differences in the results.

In a recent study, it was shown that upon systemic injection of neutrophil lysosomal extract to MPO-immunized rats a necrotizing vasculitis in the lungs and gut is developed (28). The results indicate that the release of products from activated neutrophils in the presence of anti-MPO autoantibodies may be relevant to the pathogenesis of anti-MPO-associated vasculitides.

Induction of experimental Wegener's granulomatosis

Wegener's granulomatosis (WG) is a granulomatous vasculitis that involves the upper respiratory tract, lungs and kidneys, as well as skin abnormalities, peripheral neuropathy and joint disease. WG is closely associated with the presence of ANCA, which are predominantly directed against PR-3 (24, 29). Immunization of naive BALB/c mice with purified ANCA from two patients with active WG led to the development of anti-human ANCA and anti-anti-human

ANCA (mouse ANCA) with specificity both to PR3 and to MPO, as well as anti-endothelial autoantibodies. Mouse ANCA were capable of inducing the adhesion of neutrophils to fibronectin and activating the respiratory burst in neutrophils.

Moreover, mice that were immunized with human ANCA also developed either sterile microabscesses in the lungs after 8 months, or proteinuria (but not hematuria) associated with mononuclear perivascular infiltration in the lungs and the diffuse granular deposition of immunoglobulins in the kidneys (12, 20). It was also shown that IgG-ANCA-immunized mice develop high levels of IL-4, IL-6 and TNFα, but not IL-1β, IL-2 or interferon-γ (30), suggesting that a Th2-type immune response is responsible for the initiation of experimental autoimmune lung vasculitis, similar to WG in humans. Those findings provide evidence for a direct role of ANCA in the pathogenesis of Wegener's-associated vasculitides.

Experimental Goodpasture's syndrome (GPS)

GPS is a severe autoimmune disease characterized by the triad of glomerulonephritis, pulmonary hemorrhage and anti-glomerular basement membrane (GBM) autoantibodies, directed against the non-collagenous domains (NC1) on type IV collagen (31). Abbate *et al.* (32) studied the renal and pulmonary effects of immunization with alpha3(IV) NC1 collagen in Wistar-Kyoto (WKY) rats. The immunized rats developed proteinuria, linear IgG deposition in GBM, and crescentic glomerulonephritis, the effects being dose-dependent. Pulmonary hemorrhage was detectable in 35% of immunized rats. These findings documented that glomerulonephritis and lung hemorrhage can be elicited in WKY rats by immunization with alpha3(IV) NC1. Employing our method of idiotypic immunization, we immunized naive BALB/c mice with either mouse IgG anti-NC1 monoclonal antibody, or with IgG serum fraction derived from patients with GPS. The mice developed mouse anti-NC1 antibodies of the IgG isotype (33, 34). The presence of circulating anti-NC1 antibodies coincided in some of the mice with hematuria and proteinuria, as well as pathological changes in the kidneys. The results show that specific idiotypic manipulation can induce mouse-anti NC1 autoantibodies and pathological changes resembling human GPS.

Anti-endothelial cell antibody-induced vasculitis

Anti-endothelial cell autoantibodies (AECA) have been the subject of extensive research in the last decade (35-37). AECA were detected in sera from patients with connective tissue and autoimmune diseases, such as SLE (38), rheumatoid arthritis (39), mixed connective tissue disease (39), systemic sclerosis (38) and systemic vasculitides (40, 41). Analysis of the antigens recognized by AECA showed that the antibodies are directed against a heterogeneous family of both constitutive and non-constitutive surface endothelial proteins (35). Although there are no definite conclusions concerning the clinical significance of AECA, they might well be one of the driving mechanisms for vascular injury, and one of the factors that may initiate the pathogenesis of vascular abnormalities.

Recently, we (46-48) and others (42-45) were able to demonstrate that AECA from different sources (e.g. antiphospholipid syndrome, WG, systemic sclerosis or Takayasu's arteritis) have the potential to activate endothelial cells (EC). The pre-treatment of human umbilical vein EC with AECA led to an increased secretion of IL-6 and von Willebrand factor, which are markers of EC activation, as well as to the increased ability of EC to bind human U937 monocytic cells and the increased expression of adhesion molecules. It can be hypothesized that these *in vitro* studies provide evidence for a pathogenic role of AECA in inducing vascular inflammation.

In line with this assumption, we actively immunized naive mice with purified human AECA derived from a WG patient's plasma, in an attempt to induce the production of mouse-AECA and autoimmune vasculitis in a murine model (14). IgG was purified by absorption on a PR-3 affinity column, resulting in the depletion of anti-neutrophil cytoplasmic Ab activity. The absorbed IgG fraction displayed a high titer of AECA, as evidenced by a cyto-ELISA against unfixed human umbilical vein endothelial cells. Three months after a boost injection with human AECA, the mice developed endogenous AECA, but not Abs to PR-3, CL, or DNA. Histologic examination of the lungs and kidneys revealed both lymphoid cell infiltration surrounding the arterioles and venules, and the deposition of immunoglobulins on the outer part of blood vessel walls. This experimental animal model of vasculitis, a product of our method of idiotypic manipulation, provided the first direct proof of the pathogenicity of AECA.

Autoimmune-mediated atherosclerosis

Atherosclerosis is a multi-factorial condition that results in the formation of lipid-laden lesions in the arterial system (49). The implications of the complications of atherosclerosis cannot be overemphasized, since it represents the major cause of mortality in the Western world.

The basic cellular elements that play a part, at least initially, are endothelial cells, macrophages, T lymphocytes and smooth muscle cells (49). Subsequently, the monocytes differentiate into macrophages expressing the scavenger receptor(s), which allows for the unregulated influx of oxidized lipoproteins in the cells, leading to foam cell formation (50). Oxidized LDL has attracted major interest in view of its various effects on different cellular components, attesting to its immunogenicity and probable causal effect on the progression of atherosclerosis (50). This chain of events resembles the development of chronic inflammatory conditions and even some immune-mediated diseases, and this concept has focused attention on the involvement of the immune system in atherogenesis (51-53). Indeed, the recent literature is rich in reports documenting the importance of immune mediators in the progression of the atherosclerotic process (54).

An autoimmune reaction against heat shock protein 60 (Hsp60), expressed by endothelial cells in areas that are subject to increased hemodynamic stress, was proposed as an initiating event in atherogenesis (51). Humoral and T cell-medi-

ated immune responses against Hsp60 have both been demonstrated early in the disease. In addition, several clinical studies have pointed to an association of SLE with premature atherogenesis (55). The high occurrence of APS (arterial and venous thrombosis, recurrent fetal loss and thrombocytopenia) in patients with SLE prompted the question as to whether APS might be a risk factor for early atherosclerosis.

Although no prospective controlled study has yet documented an association between antiphospholipid antibodies (aPL) and long-term atherosclerotic complications, indirect data do point to a possible linkage between the two conditions. Patients with APS secondary to SLE have recently been shown to possess markers of lipid peroxidation, indicating a pro-atherogenic potential (56). Furthermore, it was shown that aPL from APS patients induce monocyte adherence to human EC, this effect being mediated by the adhesion molecules ICAM-1, VCAM-1 and E-selectin (43, 45, 46). Bearing in mind that the initiation of human atherosclerotic lesions results from monocyte adhesion to EC, it is tempting to speculate that aPL may promote atherosclerosis *in vivo* by this mechanism (57). In order to demonstrate the pro-atherogenic effect of aPL *in vivo*, George *et al.* immunized LDL receptor-deficient mice with either β_2GPI or ovalbumin (58). All of the β_2GPI-immunized mice developed high titers of anti-β_2GPI antibodies as well as a specific lymph node proliferation to β_2GPI. Atherosclerosis was enhanced in the β_2GPI-immunized mice in comparison with ovalbumin-immunized mice. The average lesion size in the β_2GPI-immunized mice which were fed an atherogenic diet was larger than the lesions in the ovalbumin-immunized mice. The atherosclerotic plaques in the β_2GPI-immunized mice appeared to be more mature, and a denser infiltration of CD4 lymphocytes was present in the subendothelium of the aortic sinuses from this group of mice. The results of the study provided the first direct evidence for the pro-atherogenic effect of β_2GPI immunization and established a new model for immune-mediated atherosclerosis.

Similar results have been obtained using apolipoprotein E (ApoE)-deficient mice (59). In another study applying the idiotypic manipulation method, George *et al*. assessed the effect of immunization with aCL antibodies (i.e. Ab1, leading to the production of mouse aCL-Ab3) on the progression of atherosclerosis. Two groups of LDL-receptor knockout mice (LDL-RKO) were immunized with IgG purified from the serum of an APS patient or with normal human IgG, respectively. The aCL-immunized mice developed high titers of self-aCL compared with the normal human IgG-immunized mice. The extent of fatty streak formation was significantly higher in the aCL-immunized mice in comparison with the human IgG-injected mice. The results of the study show that mouse aCL induced by immunization with human aCL from an APS patient enhances atherogenesis in LDL-RKO mice and further support the role of aPL in the development of atherosclerosis in patients with APS (60).

In another study, C57BL/6J mice were immunized with recombinant heat shock protein-65 (HSP-65) and HSP-65-rich *Mycobacterium tuberculosis* (MT). A rapid cellular immune response to HSP-65 was evident in the immunized mice, accompanied by enhanced early atherosclerosis. Immunohistochemical analysis of atherosclerotic lesions from the immunized mice revealed infiltration of CD4 lymphocytes compared with the relatively lymphocyte-poor lesions in controls. This model, which supports the involvement of HSP-65 in atherogenesis, furnishes a valuable tool to study the role of the immune system in atherogenesis (61).

Conclusion

Animal models are of great value in the evaluation of pathogenic mechanisms, as well as of novel and experimental treatments which cannot be tested directly on patients. We have summarized a spectrum of animal models for vascular diseases induced by the idiotypic immunization method, among them ANCA-associated vasculitis, experimental Goodpasture's syndrome, AECA-associated vasculitis and immune-mediated atherosclerosis. These experimental models might serve as a valuable tool for assessing pathogenic mechanisms, as well as novel modalities of treatment, in different stages of these diseases.

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