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REVIEW

Anti-endothelial cell antibodies: only for scientists or for clinicians too?

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INTRODUCTION

Antibodies directed against vascular endothelium are now recognized as a common serological feature in several diseases characterized by immune-mediated vascular damage [1]. Several questions have been raised regarding the specificity, clinical value and potential pathogenic role *in vivo* of these new autoantibodies.

DO AUTOANTIBODIES AGAINST VASCULAR ENDOTHELIUM EXIST OR NOT?

The ability to culture human endothelial cells (EC) *in vitro* has led to the development of several assays to detect autoantibodies against vascular endothelium (AECA). Several different techniques, including standard microcytotoxicity, cellular ELISA or radioimmunoassays, cytofluorimetry, Western blotting analysis on cell extracts or immunoprecipitation of radiolabelled surface endothelial proteins [1,2], have been used in the detection of AECA. These studies have confirmed the original observations in the early 1970s, when these autoantibodies were first described in sera from patients with systemic autoimmune diseases using indirect immunofluorescence assays with mouse kidney sections [3,4]. In addition, antibodies against AECA idiotypes have also been described in intravenous immunoglobulin (IVIg) preparations, further supporting the occurrence of these autoantibodies and suggesting a potential mechanism by which IVIg can be effective in treating AECA-positive vasculitis, such as Kawasaki disease [5].

IS ANTI-ENDOTHELIAL REACTIVITY DUE TO SPECIFIC ANTIBODY BINDING?

Binding specificity

Immunoglobulin deposition on endothelial monolayers has been reproduced *in vitro* using purified whole immunoglobulins as well as by $F(ab')_2$ fragments, but not by the Fc portion, suggesting that AECA binding is antibody-specific and in addition is unaffected by immune complex removal. IgG, IgM and IgA isotypes have all been described in a variety of diseases, including IgA nephropathy [1].

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Antigen specificity

Attempts to characterize endothelial antigens using immunoprecipitation of radiolabelled endothelial cell surface proteins have shown reactivity with a number of proteins ranging in size from 25 to 200 kD [6]. The number of antigens seen using immunoblotting is even greater, and may be explained by possible contamination of surface proteins with nuclear and cytoplasmic components when the endothelial cells were disrupted. Nevertheless, these studies suggest that AECA represent a heterogeneous family of antibodies reacting with a variety of different structures on endothelial cells [7,8].

Antigens recognized by AECA seem to be represented mostly by constitutive proteins present on resting endothelial membranes. In most of the investigated disorders, endothelial cell activation by cytokines or by other agonists does not affect the expression of the epitopes suitable for AECA. The exception to this is the cytokine-induced modulation of endothelial binding and cytotoxicity seen in Kawasaki disease and in haemolytic uraemic syndrome [1].

In addition to constitutive proteins, at least in systemic lupus erythematosus (SLE) sera, the AECA assay is able to detect immunoglobulin bound to other molecules such as β_2-glycoprotein I (β_2-GPI; the plasma cofactor for anti-phospholipid antibodies) and DNA and DNA/histone complexes which adhere to the endothelial surface through their electrical charges [9,10]. These recent data provide an attractive hypothesis for one of the potential pathogenic mechanisms involved in lupus vasculitis. Further evidence of the complexity of the different antibodies detectable by the whole cell ELISA comes from the observation that sera from systemic autoimmune diseases also contain subpopulations of antibodies against extracellular endothelial matrix components [11].

Cell specificity

AECA are capable of binding endothelium from a variety of sources such as arteries, veins, human and murine endothelial cell lines. Most studies have employed human umbilical vein endothelial cells (HUVEC) as substrate; however, HUVEC may not be entirely representative of the antigens involved in *in vivo* organ-specific processes. For example, the use of microvascular endothelial cells resulted in a higher prevalence of AECA in Behçet's disease compared with HUVEC (reviewed in [12]).

Finally, AECA are not endothelial cell-specific, since several groups have shown cross-reactivity with other cells such as

fibroblasts and, at least partially, with peripheral blood mononuclear cells (PBMC) [1].

ARE AECA SIMPLY AN EPIPHENOMENON?

The absence of AECA in diseases such as mixed essential cryoglobulinaemia where vascular damage is clearly mediated by other immune effectors, suggests that these antibodies represent a primary event rather than merely a secondary immune response against determinants exposed in the course of the vascular inflammatory process [13]. In addition, AECA titres in primary and secondary vasculitis do not correlate with the total amount of serum immunoglobulins [1] and AECA are absent in sera from patients with signs of polyclonal B cell activation such as HIV^+ patients with persistent generalized lymphadenopathy [13]. These factors militate against the idea that the fall in AECA levels with therapy in SLE and Wegener's granulomatosis (WG) patients is due simply to a non-selective down-regulation of autoantibody production.

DO AECA HAVE CLINICAL UTILITY?

Disease associations

AECA have been detected in a wide variety of immune-mediated diseases. The largest group includes the vasculitides, both primary and secondary to connective tissue diseases. Although AECA are commonly detectable in the systemic vasculitides, they do not display any disease specificity. The highest prevalences have been reported in WG, microscopic polyangiitis (MPA), Kawasaki disease, lupus nephritis and the anti-phospholipid syndrome (APS) [1].

Correlation with disease activity and clinical features

There is a consensus that AECA titres correlate with disease activity. For example, AECA in Kawasaki disease, although not associated with any particular clinical manifestation (even coronary aneurysms), were detectable only in the active states but not during remission. In WG and MPA, AECA titres decreased during therapy induced remission, correlated with other clinical and laboratory parameters of disease activity, as well as endothelial damage, and were able to predict relapses [14]. Cines *et al.* [15] reported AECA in active disease and D'Cruz *et al.* [16] found a higher prevalence in lupus nephritis with correlations between AECA levels and renal activity scores. In contrast, Van der Zee *et al.* [7] noticed only a correlation between AECA and skin or joint involvement. However, immunoblot analysis of the same sera revealed that antibodies against endothelial proteins of 38, 41 and 150 kD were associated with renal involvement. A positive correlation was also found between the presence of the antibodies and severity of vascular lesions in systemic sclerosis (reviewed in [12]). Finally, sera from patients with accelerated or chronic allograft rejections displayed anti-endothelial activity which was not due to antibodies directed against HLA molecules [1].

ARE AECA UNDER GENETIC CONTROL?

An association between AECA and HLA-DR7 and DQw2 as well as a weak link with DPB1*1401 and a possible protective effect of DPB1*0401 have recently been reported [17]; the same authors suggested that the association of these DPβ alleles was independent of linkage to the DR7 and DQw2-associated susceptibility antigens. The same group has previously reported an association between anticardiolipin antibodies and the DPB1*1401 allele [18], and this is reminiscent of the possible association between AECA and anticardiolipin antibodies in SLE patients. Taken as a whole, these studies suggest a possible role for the MHC in controlling AECA production in these patients.

ARE AECA PATHOGENIC AUTOANTIBODIES?

Circulating antibodies, such as AECA, reacting against available surface antigens could well be involved in pathogenic mechanisms. Although AECA can fix complement *in vitro* [15,19], most authors have been unable to confirm direct or complement-mediated cytotoxicity on endothelial monolayers. However, some but not all AECA-positive sera can mediate antibody-dependent cellular cytotoxicity (ADCC). In WG and MPA sera, this lytic activity was mediated by IgG fractions and natural killer (NK) cells, but required high effector/target ratios. Even cytokine activation did not significantly affect susceptibility to ADCC lysis [13,20]. These findings suggest that ADCC is probably not a key mechanism in vascular damage. An exception may be the complement-dependent cytotoxicity displayed by AECA in acute Kawasaki disease on cytokine-activated but not on resting EC [1].

Rather than being directly cytotoxic, AECA could affect endothelium by modifying some of its complex functional processes. For example, IgG fractions from patients with anti-phospholipid (aPL)-associated thrombosis influenced endothelial prostacyclin production, raising the possibility that aPL directly affects the balance between prostacyclin and thromboxane production [21]. Similarly, it has been suggested that immunoglobulin deposition on the endothelial membrane could result in endothelial activation. The finding that β_2-GPI mediates aPL binding to the endothelial surface [22–24] lends considerable support to this hypothesis. β_2-GPI is a cationic protein that binds to endothelial surfaces and offers suitable epitopes to both β_2-GPI-dependent aPL and anti-β_2-GPI antibodies. In fact, β_2-GPI can be recognized by aPL-positive sera which appear to mimic endothelial cell reactivity. Accordingly, affinity-purified anti-β_2-GPI antibodies and MoAbs recognizing cryptic epitopes expressed on the β_2-GPI molecule after its complexing with phospholipids, bind to endothelial monolayers and induce cell activation. This activation was evidenced by increased prostacyclin metabolism, the production of proinflammatory cytokines (IL-6), and the up-regulation of adhesion molecules [22,25]. Endothelial activation might favour the appearance of a procoagulant endothelial phenotype which represents a potential pathogenic mechanism for the thrombotic diathesis in the anti-phospholipid syndrome.

Endothelial activation has also been reported after incubation with IgG from primary vasculitides such as WG and MPA or from a patient suffering from systemic sclerosis (reviewed in [12]). In particular, AECA-IgG from WG can induce dramatic up-regulation of adhesion molecules and increased secretion of proinflammatory (IL-1β, IL-6) and chemoattractant (IL-8, MCP-1) cytokines [26]. Taken together, these findings strongly support a pivotal role for AECA in vascular damage by attracting leucocytes to the inflammatory site, facilitating not only their adhesion to the inflamed vessel walls, but also their extravascular migration and granuloma formation.

ARE THERE EXPERIMENTAL ANIMAL MODELS OF AECA-ASSOCIATED VASCULAR DAMAGE?

AECA have been detected in sera from mice with spontaneous SLE (MRL *lpr/lpr*) [24] and with lupus-like disease experimentally induced by idiotypic manipulation (reviewed in [12]). The occurrence of AECA in these animal models mirrors AECA reactivity in human disease.

Murine AECA have also been induced in naive mice injected with whole IgG fractions from a WG patient positive both for ANCA and AECA. The mice developed antibodies against ANCA antigens but also IgG-AECA that immunoprecipitated surface endothelial proteins quite comparable to those recognized by human WG sera. Interestingly, the same animals displayed pulmonary and renal lesions [26].

More recently, Damjanovic *et al.* [27] injected naive mice with human AECA-IgG fractions absorbed for ANCA activity and demonstrated the appearance of murine IgG AECA after 3 months, confirming that AECA were also under idiotypic control. Histological examination of the lungs and kidneys of these animals with AECA revealed perivascular lymphoid cell infiltration and deposition of immunoglobulins in the outer part of vessel. These data support the previous findings that guinea pigs immunized with an endothelial membrane extract developed AECA and histological signs of central nervous system (CNS) and renal vasculitis, together with deposition of radiolabelled AECA [28]. Taken together, these data support a direct pathogenic role for AECA in inducing vascular damage in these animal models.

WHAT IS THE FUTURE FOR AECA?

The recent rapid expansion in this field has prompted several groups to demonstrate a potential pathogenic role for AECA both in *in vitro* and *in vivo* experimental models. Such reports are in line with the clinical associations between AECA and autoimmune vasculitis. However, in spite of this intense interest, the most urgent priority is the standardization of an accurate assay for the detection of AECA [29]. Once this has been achieved, AECA could then be properly evaluated as a diagnostic and prognostic tool. A major step towards this goal will be the definition of the endothelial antigens involved. This could be facilitated by the use of endothelial DNA libraries or endothelial cell lines expressing disease-specific antigens. In addition, human monoclonal AECA could help in the analysis of the V_H regions preferentially utilized and in obtaining anti-idiotypic antibodies as suitable reagents to monitor the presence of AECA in biological samples.

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