

Anti-Endothelial Cell Antibodies and Autoimmune Diseases

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Introduction

Antibodies reacting with endothelial structures (AECA) have been detected in autoimmune diseases by standard indirect immunofluorescence, by solid-phase radio- or enzymatic-cell binding immunoassays and more recently by immunoblotting or immunoprecipitation and sodium dodecyl sulfate (SDS)-analysis of the precipitated proteins. Different endothelial proteins, including cell-surface constituents, are recognized by AECA; the antibodies, however, are not endothelium specific since they can crossreact, at least in part, with other cell types. They play a probable pathogenetic role in in vivo vessel wall damage as supported by different in vitro models of direct and complement- or cell-mediated cytotoxicity. AECA can be detectable in different unrelated autoimmune vasculitis and their presence generally correlates with disease activity. The diagnostic value of AECA could be useful in diseases lacking of other specific serological markers such as in Kawasaki syndrome or in idiopathic forms of vasculitis.

Involvement of Endothelium in Autoimmune Diseases

Over the last two decades, it has become increasingly clear that vascular endothelium plays a key role in the pathogenesis of vascular inflammation in systemic autoimmune diseases. In fact, because

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of its wide anatomical diffusion and because of its continuous contact with circulating immune effectors, endothelium represents an accessible and potential target for an immune-mediated autoaggression. A variety of mechanisms of vascular pathology is implicated in autoimmune diseases: deposition of circulating immune complexes, cell-mediated damage, reactivity with autoantibodies, and direct effect of different cytokines (1). In any case, endothelial cells should not be considered only as passive targets since it is now clear that they also play an active role in inflammatory and immune processes. In fact, endothelial cells can promote inflammation and influence immune response (2,3) through different ways:

1. Leukocyte adhesion, promoted by increased expression of endothelial adhesion molecules (4,5);
2. Expression of class I and II HLA molecules, allowing endothelial cells to act as antigen-presenting cells (6–10) and as target for cytotoxic T-lymphocytes;
3. Cytokine production (11);
4. Expression of procoagulant activity (12); and
5. Changes in cell morphology leading to increased vascular permeability (13).

More recently, antibodies reacting directly with endothelial cells were also reported. Anti-endothelial cell antibodies (AECA) were demonstrated in a variety of autoimmune diseases, and particular efforts have been made to determine whether these antibodies may be pathogenic.

Assays for AECA Detection

Antibodies reacting with endothelial structures were first described independently by Lindqvist and Osterland (14) and Tan and Pearson (15). These authors used an indirect immunofluorescent technique on frozen mouse kidney sections as substrate and detected AECA in sera from patients with different systemic autoimmune diseases.

More recently, the possibility to isolate and to culture human endothelial cells *in vitro* (16) led to the development of sensitive cell-surface radioimmunoassays (RIAs) (17) or enzyme-linked immunosorbent assays (ELISAs) (18). By these techniques, several authors described AECA in sera from patients affected by different immune-mediated vascular damage, such as systemic autoimmune diseases (17–21), Berger's disease (22), Kawasaki syndrome (KS), hemolytic uremic syndrome (HUS) (23,24), and primary vasculitis (25–27).

Most of the AECA detected by these assays belonged to the IgG and IgM subclasses and the binding to endothelial cell membrane was mediated by FAb₂ fragments (21,28,29). Moreover, in 1989

Brasile et al. described the presence of antibodies against endothelial cells in primary and secondary systemic vasculitis by using a standard complement dependent microcytotoxicity assay (30).

The presence of circulating autoantibodies against endothelial components has been confirmed also by biochemical methods. Antibodies against solubilized endothelial cell extract were detected by immunoblotting technique in sera from patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) complicated by vasculitis (31,32), and in sera from patients with thrombotic microangiopathies (thrombotic thrombocytopenic purpura and HUS) (33). The use of immunoblot techniques displays some advantages in comparison to the previous assays, offering the opportunity to define the specific molecular weight or the isoelectric point of the endothelial antigens, as well as providing the potential for antigen purification and sequencing.

Although AECA could be detectable by both ELISA and immunoblotting in the majority of sera, frequently, the results between the two assays differed (31,32). This fact was related to the different sensitivity between the tests or to the changes in antigen presentation during membrane preparation. Furthermore, McCrae et al. and Del Papa et al. independently set up an immunoprecipitation assay to detect antibodies reacting with selectively radiolabelled surface membrane proteins from human umbilical vein endothelial cells (34,35). Del Papa et al. (35) showed that electrophoretic analysis of the immunoprecipitated proteins confirmed the presence of AECA in Wegener's granulomatosis (WG) and SLE sera able to precipitate surface endothelial proteins from 200–25 kDa. The number of the immunoprecipitated bands was smaller (9 bands) than that previously reported (19 bands) by Van der Zee et al. (31) using a crude cell-membrane extract. This difference was related to the selective involvement of surface proteins in the last assay, avoiding any contamination with endothelial intracellular structures, against which a variety of autoantibodies can be present at least in SLE sera.

Disorders Associated with AECA

AECA have been detected in different, unrelated immune-mediated diseases (Table 1). The largest group includes patients with autoimmune vasculitis, both primary and secondary to systemic autoimmune diseases (36). Different studies in fact confirmed the presence of AECA in primary vasculitis, such as WG and micropolyarteritis (MPA), with a prevalence up to 80% (25–27,29,30). Moreover, IgG-AECA were reported to occur in 0–88% of SLE patients (17,18,21,28,31,35,37,38) and also in sera of patients suffering from antiphospholipid syndrome (28,34,39,40).

Table 1
Different Pathological Conditions
in which AECA Have Been Reported

Disease	Prevalence	Refs.
SLE	0–88%	17,18,21,28,31,35,37,38,48
RA with vasculitis	80%	32,38
Mixed connective tissue disease	45% (20/44)	41
Scleroderma	20–30%	18,21,42
Polymiositis/dermatomyositis	44% (8/18)	60
WG	50–80%	25–27,29,30
PAPS	60–63%	34,39,40
Behçet disease	18–33%	61–63
Idiopathic retinal vasculitis	35% (7/20)	63
KS	65% (21/32)	64
Haemolytic uraemic syndrome	93% (13/14)	24
Thrombotic thrombocytopenic purpura	100% (3/3)	65
Heparin-associated thrombocytopenia	100% (27/27)	58
HLA-matched graft rejection	—	43,66–68
Inflammatory bowel disease	25–43%	69,70
Insulin-dependent diabetes mellitus	up to 34%	71
Multiple sclerosis	23% (7/32)	72
Immune-mediated hypoparathyroidism	100% (6/6)	73
Episodic angioedema/hypereosinophilia	100% (3/3)	74
Acute preeclampsia	50% (9/18)	75
Berger's disease	32% (26/72)	22
Viral infections	up to 18%	76
Rocky Mountain Spotted Fever	50% (7/14)	77

AECA were reported in RA complicated by vasculitis (32,38), in mixed connective tissue disease (41), and in progressive systemic sclerosis (18,21,42). Studies undertaken in KS and HUS are especially interesting since AECA were demonstrated to be related to cytokine modulation of endothelial cells.

Finally, the third largest group in which AECA were studied includes allograft rejections. AECA directed against non-HLA molecules on graft endothelium have been detected in a proportion of allograft recipients who developed either accelerated or chronic graft rejection, suggesting that antibodies reactive with the endothelium may take part in graft rejection (43–47).

Correlation with Disease Activity

The majority of the studies on the occurrence of AECA in autoimmune vasculitis (both primary and secondary to systemic autoimmune diseases) reported that the antibodies correlated with disease activity.

Studies in acute KS demonstrated the presence of both IgG and IgM AECA. Although AECA titers did not correlate with any particular clinical manifestation of the syndrome, in particular with the appearance of coronary artery aneurysms, the anti-endothelial activity was no longer detectable during convalescent phase (23,24). In primary vasculitis, such as WG and MPA, AECA titers decreased during therapy-induced remission and correlated with other clinical or laboratory parameters of disease activity and endothelial damage such as plasma von Willebrand Factor (vWF) levels (25,26).

A comparable correlation was not so clear in patients with SLE-associated vasculitis. Actually, Cines et al. (17) reported the presence of AECA in sera from patients with active disease, and, more recently, D'Cruz et al. (48) found that SLE patients with renal involvement displayed higher prevalence of AECA in comparison with patients without nephropathy. The authors suggested that AECA could be a potential marker for nephritis and vasculitis. On the contrary, Van der Zee et al. (31) found a significant correlation only with skin and joint involvement. However, in the same SLE sera, antibodies reacting with endothelial antigens of 38, 41, and 150 kDa apparently correlated with renal involvement (31).

Characterization of the Endothelial Antigens Recognized by AECA

In spite of the well-documented presence of AECA in different pathological conditions, little is still known about the antigens recognized by these antibodies. Different studies showed that AECA represent a heterogeneous group of autoantibodies reacting with a variety of different structures on endothelial surface (21,31,32,35,40). Antigenic epitopes are not specific for endothelial cells only as demonstrated by absorption studies with both fibroblasts and peripheral blood mononuclear cells (17,21,30,31,38,49). Probably different antigens are recognized on different cells or similar epitopes are shared by a number of cells. It is generally accepted that AECA activity does not involve HLA class I and II or ABO blood group antigens (25-27,29).

AECA are unrelated to other circulating autoantibodies, including antinuclear antibodies, antibodies to extractable nuclear antigens, or rheumatoid factor (35,38,50). In addition, AECA represent a separate entity from the antineutrophil cytoplasm antibodies (ANCA) frequently detectable in sera from primary vasculitis patients (25-27). On the contrary, an apparent partial cross-reactivity between endothelial structures and anti-DNA antibodies was found in SLE sera (51,52). This finding, however, was related to

the presence of anti-DNA antibodies that bind to endothelial cells through DNA or DNA/histone molecules adhered to the membranes (53). In addition, previous studies supported the hypothesis that antibodies directed against negatively charged phospholipids could be part of the anti-endothelial cell activity in certain antiphospholipid (aPL) antibody-positive SLE sera (28). Utilizing immunoprecipitation experiments with labeled endothelial surface proteins, McCrae et al. observed that AECA-positive sera from patients affected by primary antiphospholipid syndrome (PAPS) reproducibly precipitated a protein of about 70 kDa (34). In another study, Del Papa et al. (35,40) demonstrated that AECA-positive sera from patients with antiphospholipid syndrome (APS) reacted with a variety of endothelial surface proteins displaying different molecular weights. The authors also demonstrated that beta-2 glycoprotein I (β 2GPI), a plasma cofactor involved in the binding between phospholipid and aPL antibodies, was able to adhere to the endothelial membranes. It is likely that AECA and aPL antibodies represent two distinct families of antibodies, which however can be frequently associated in patients with APS (39,40). Besides occasional aPL antibodies recognizing endothelial phospholipids (28,54), most of the AECA in these patients seems to react with different cell-membrane proteins, among which β 2GPI could also play a role in the binding (40).

Using immunoblotting techniques with endothelial cell extracts, two groups identified different endothelial proteins recognized by AECA-positive sera from patients with both primary and secondary autoimmune vasculitis (31,32,35,40). These studies showed that AECA reacted with a wide spectrum of surface molecules with different molecular weights, ranging in size from 200–15 kDa. Particularly, Del Papa et al. reported that WG sera immunoprecipitated five different surface proteins (from 180–25 kDa) with a pattern more homogeneous than that shown by SLE sera. In addition to the bands shared in common, some endothelial proteins were exclusively precipitated by WG or alternatively by SLE sera, suggesting that in primary and secondary vasculitis different specific endothelial antigens could be involved in the autoimmune aggression to vessel structures.

Furthermore, experiments carried out with endothelial cells extensively washed with ethylenediaminetetraacetic acid (EDTA) buffer showed that WG-AECA-positive sera retained their reactivity, suggesting that integral constituents of the endothelial membranes were recognized. In contrast, in SLE patients, AECA were also directed against proteins absorbed on the cell surface (35).

The endothelial antigen/antibody system involved in KS and HUS appears to be characterized by its peculiar susceptibility to

cytokine modulation. In fact, AECA in KS or HUS sera were cytotoxic only for IL-1 β , TNF- α , or IFN- γ treated but not for resting endothelial cells (23,24,55,56). This finding is not surprising, keeping in mind that many of the actions of these cytokines on endothelial cells seem to involve modulation of the expression of surface molecules. In contrast, AECA-positive sera from WG and MPA patients recognized determinants constitutively present on endothelial surface that were not upregulated (or only partially upregulated) by cytokine treatment (27,29,49).

Possible Pathogenetic Role of AECA

In spite of the lack of an absolute specificity for endothelial cells as well as their lack of disease specificity, the above-mentioned demonstration of AECA ability to react with endothelial surface structures further supports a pathogenetic role for these autoantibodies.

The absence of AECA in diseases with vascular damage mediated by other immune effectors seems to suggest that the anti-endothelial antibodies represent a primary event rather than a mere immune response against determinants exposed as a consequence of the vascular inflammatory process (25,29). In addition, anti-endothelial activity is absent in AIDS patients with polyclonal B-cell activation (29), and there is no correlation between AECA titers and total serum Ig levels both in primary and secondary vasculitis (25,27,48), suggesting that their presence is related to a specific antigen-driven immune response.

Several studies investigated the possible pathogenetic role of these antibodies in inducing the cell wall damage. Though initially Cines et al. demonstrated AECA to be able to fix complement in SLE sera (17), only Brasile et al. (30) were able to confirm this finding. In fact, the majority of sera from SLE or scleroderma patients did not show a cytotoxic endothelial damage either alone or after addition of fresh complement or mononuclear cells (19,20). Moreover, no evidence for direct or complement-mediated cytotoxicity in AECA-positive sera from patients with primary vasculitis was found (25–27,57). The absence of any direct cytotoxic activity in AECA-positive sera was also confirmed by vWF release experiments: In fact, endothelial cells cultured in the presence of purified IgG from high positive AECA sera did not release significant amounts of vWF in culture supernatants (49). Nevertheless, when endothelial monolayers, sensitized by WG-AECA-positive sera or by their IgG fractions, were incubated with normal Fc gamma-receptor positive mononuclear cells, an antibody dependent cellular cytotoxicity could be found in some but not in all the WG sera studied (27,29). The lysis was medi-

ated by NK-cells, required high effector/target ratios, and was not increased by cytokine activation of endothelial cell monolayers (29).

Interestingly, AECA in KS display a complement-dependent endothelial cytotoxicity on cytokine-activated (IFN- γ , IL-1 α or β , and TNF- α) but not on resting endothelial cells. The target antigens induced by IFN- γ seem to be quite different from those induced by IL-1 or TNF as suggested by the different antibody isotype involved, the different time course of antigen expression, and the absorption experiments (23,24,58). In contrast with the finding reported in KS, AECA detected in patients with HUS and thrombotic thrombocytopenic purpura showed a quite peculiar pattern of endothelial cytotoxicity: These antibodies, in fact, lysed resting endothelial cells but not IFN- γ -activated endothelial cells. In other words, IFN- γ seems to be able to induce a specific loss, or an alteration in structure or accessibility of the endothelial cell-surface molecules recognized by the antibodies present in HUS patients (24). The production of antibodies directed toward cytokine-induced endothelial neo-antigens suggests that these patients generate an immune response directed to their abnormally stimulated vascular endothelium.

Altogether these *in vitro* findings seem to support an *in vivo* pathogenetic role for AECA in damaging vessel structures. It could be useful to speculate that other changes in endothelial cell functions, rather than cell killing, may contribute to the pathogenesis of vessel wall injury in autoimmune vasculitis. AEC, for example, may be a cofactor, together with cytokines, in increasing endothelial adhesivity for leukocytes (59) or in altering the hemostatic balance of endothelial cells to a prothrombotic state (56). Besides causing vasculitis and thrombosis, AECA may also affect vascular permeability, angiogenesis, and lipid metabolism.

Conclusion

AECA represent a new family of autoantibodies detectable in systemic autoimmune diseases. Different findings support their potential importance both from a diagnostic and a prognostic point of view:

1. AECA do not seem to be a mere epiphenomenon;
2. Are not related to a polyclonal B-cell activation;
3. React with surface endothelial antigens;
4. Correlate with disease activity;
5. In some cases behave as an indirect marker of *in vivo* endothelial damage;
6. Correlate with disease activity; and
7. *In vitro* experiments support a possible pathogenetic role in sustaining autoimmune vasculitis.

On the other hand, AECA do not display an absolute cell specificity, being crossreactive, at least in part, with other cell types and lack of disease specificity. Up to now, AECA determination are still matter of research but their clinical usefulness in autoimmune diseases could increase when simpler assays for the detection will be available.

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