Antibodies Directed to Sonicated Human Endothelial Cells in Patients with Vasculitis

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Dear Sir,

Anti-endothelial cell antibodies (AECA) have been described in several autoimmune diseases, such as vasculitis [1], lupus [2], hemolytic-uremic syndrome [3], and Kawasaki disease [4]. In lupus they seem to predict nephritic or vasculitic damage. In other conditions, their clinical significance remains unknown.

AECA can be detected by direct staining on tissue biopsy sections, or enzyme-linked immunosorbent assay (ELISA) in confluent cell-coated plates. Other authors [5] developed assays for the detection of AECA directed to an endothelial membrane preparation obtained from human umbilical vein endothelial cells (HUVEC) by sonication and centrifugation on a Percoll gradient.

We have tested sera of patients with vasculitis for the presence of AECA directed to cytoplasmic antigens of HUVEC, obtained by sonication.

HUVEC, isolated from human umbilical vein perfused with 5,500 U collagenase (Sigma, St. Louis, Mo., USA) were grown in 95% air, 5% CO₂ at 37 °C in culture medium containing: M199 (Gibco, Paisley, Scotland), 20% fetal bovine serum (FBS; Gibco), penicillin 100 U/ml (Sigma), streptomycin 100 µg/ml (Sigma), amphotericin 0.25 µg/ml (Sigma), 25 mM Hepes (Gibco), endothelial cell growth factor (Boehringer Mannheim, Germany), and heparin 20 U/ml (Parke-Davis, Lainate, Miss., USA). Cells were grown to confluency, then passaged using a 0.5-mg/ml trypsin-0.25-mmol EDTA solution (Gibco). Cells were frozen in 10% DMSO FBS when they reached the 6th to 7th passage.

NHS MGN ANCA +ve ANCA -ve
Fig. 1. NHS = Normal human sera; MGN = membranous glomerulonephritis; ANCA+ve = ANCA-positive sera; ANCA-ve = ANCA-negative sera; O.D. = optical density.

We tested 17 ANCA-positive and 8 ANCA-negative sera of patients with vascul-
HUVEC were positive for factor VIII antigen.

Cells were sonicated by 20 one-minute bursts in ice cooling, centrifuged at 8,000 g for 30 min at 4 ºC and the supernatant was then used for coating 96-well plates for ELISA. After incubation with 1/5 diluted KAKGER

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litis, 10 normal human sera (NHS) and 7 membranous glomerulonephritis (MGN) patient sera. The upper normal limit was considered the 90th percentile of NHS.

The results are shown in figure 1. Seven of 17 ANCA-positive sera showed the presence of AECA, while neither ANCA-negative or MGN patient sera had AECA. Among these 7 sera, 4 were positive for anti-myeloperoxidase antibodies, and 3 for anti-proteinase 3 antibodies. Mean values of ANCA-positive sera were significantly different from those of diseased sample controls (ANCA-negative sera of patients with vasculitis and sera of patients with MGN, p < 0.05).

AECA are frequently found in vasculitis, but they do not constitute a specific marker of this disease, differently from ANCA: Savage et al. [1] reported a prevalence of 59% of IgG-AECA in 168 patients with vasculitis.

Many hypotheses have been advanced concerning the possible pathogenetic role of AECA, such as induction of complement activation [4], interference with the anticoagulant properties of endothelial cells [6], engagement of cytotoxic cells [7], and expression of adhesion molecules [7].

The antigenic specificity of AECA probably varies in different diseases, and membrane antigens seem to be involved, for example, in lupus. Our test allows the detection of AECA directed to endothelial cell cytoplasmic antigens. Further studies are currently underway to correlate AECA to clinical presentation, vascular injury and response to therapy.

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References


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