



# Classification of anti-endothelial cell antibodies into antibodies against microvascular and macrovascular endothelial cells: The pathogenic and diagnostic implications\*

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**A**ntibodies that react with endothelial cell (EC) structures (known as anti-endothelial cell antibodies [AECA]) were first reported in the early 1970s during an immunohistochemical study of kidney biopsy specimens. The examined sera were from patients with various rheumatic diseases, including systemic lupus erythematosus (SLE) and scleroderma.<sup>1,2</sup> Since then, several methodologic approaches have demonstrated the existence and potential pathogenic role of AECA in a wide variety of inflammatory diseases (for review, see ref. 3). The AECA bind to different structures on endothelial membranes, mainly through the F(ab)<sub>2</sub> portion of the immunoglobulin.<sup>3</sup> IgG, IgM, and IgA isotypes of those antibodies have been reported. The EC target antigens for AECA have not yet been defined, but it is clear that there are likely to be multiple target antigens.<sup>4-7</sup> Accordingly, sera positive for AECA have been shown to display a broad reactivity against EC obtained from different human anatomic sources: from large arterial (aorta) or venous (umbilical cord vein, saphenous vein) vessels as well as from small vessels such as renal, skin, omental, and brain microvasculature.<sup>8-10</sup> In addition, AECA are apparently not species specific, since they cross-react with human, bovine, and murine EC (for review, see refs. 3 and 11).

In this respect, one can speculate that AECA are non-specific antibodies that are directed against endothelial

antigens and commonly expressed on the majority of vascular tissue. Based on our previous observations that AECA in large-vessel diseases such as *Takayasu's arteritis* (TA) bind to and activate *macrovascular human umbilical vein endothelial cells* (HUVEC), and not microvascular EC,<sup>12</sup> we advocate the attractive hypothesis that AECA from different sources recognize different types of EC target molecules, which is correlated with the origin of the disease. Therefore, classification of AECA into antibodies that are directed against microvascular and macrovascular EC should shed more light on this complex group of antibodies. This lecture will summarize the results of some of our previously published studies as well as the current literature on this subject, which would support a rationale for proposed classification of AECA.

## ■ CLASSIFICATION OF AECA AS ANTIBODIES AGAINST MICROVASCULAR AND MACROVASCULAR EC ANCA-positive necrotizing and crescentic glomerulonephritis

The first reports of disease-specific vascular bed involvement came from studies on autoantigens of ANCA in necrotizing and crescent glomerulonephritis (NCGN).<sup>13</sup> ANCA-positive sera of patients with NCGN reacted with glycoproteins from a membrane preparation of polymorphonuclear neutrophil granulocytes, designated as gp170/80-110 and verified to be identical with human lysosomal-associated membrane protein 2 (h-lamp-2). Unexpectedly, these sera also cross-reacted with 130-kD EC membrane glycoprotein (gp130) of the renal microvasculature. Gp130 is also present on the surface of EC of intestinal capillaries and placental capillaries but not on EC of arteries and arterioles.<sup>13</sup> Accordingly, both monoclonal antibody against gp170/80-110 and rabbit anti-gp130 failed to bind unstimulated and IL-1-treated HUVEC. Interestingly, 14 of 16 patients with NCGN had IgG specific for gp130 and gp170/80-110. The relation between gp130 and h-lamp-2 has not been established yet. A possible explanation is that the 130-kD antigen shares one or several epitopes with h-lamp-2. It is also possible

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**TABLE 1**  
PREVALENCE OF ANTI-ENDOTHELIAL CELL ANTIBODIES AMONG DIFFERENT IMMUNOLOGICALLY MEDIATED DISEASES

| Disease   | Prevalence (%) | References*       |
|---|----------------|-------------------|
| <b>Primary autoimmune vasculitis</b>              |                |                   |
| Wegener's granulomatosis/microscopic polyangiitis | 55-80          | 8, 9, 17, 19, 20  |
| Kawasaki disease                                  | Up to 72       | 28-31             |
| Takayasu's arteritis                              | 95             | 32                |
| Giant-cell arteritis                              | Up to 50       | 11                |
| Idiopathic retinal vasculitis                     | 35             | 33                |
| Behçet's disease                                  | Up to 50       | 10                |
| Thromboangiitis obliterans                        | 25-36          | 11, 34            |
| Churg-Strauss disease                             | 50             | 11                |
| <b>Systemic autoimmune diseases</b>               |                |                   |
| Systemic lupus erythematosus                      | Up to 80       | 4, 14, 17, 35, 36 |
| Antiphospholipid syndrome                         | 64             | 40                |
| Rheumatoid arthritis with vasculitis              | Up to 65       | 17, 45, 46        |
| Rheumatoid arthritis without vasculitis           | Up to 30       | 17, 45, 46        |
| Systemic sclerosis                                | 20-80          | 6, 47, 48         |
| Mixed connective tissue disease                   | 45             | 17                |
| Polymyositis/dermatomyositis                      | 44             | 53                |
| <b>Transplantation</b>                            |                |                   |
| Heart and kidney allografts                       | Up to 71       | 15, 54, 55        |
| <b>Miscellaneous</b>                              |                |                   |
| Inflammatory bowel disease                        | Up to 55       | 11, 62-64         |
| Hyperprolactinemia in women                       | 76             | 65                |
| Hemolytic uremic syndrome                         | 93             | 66                |
| Thrombotic thrombocytopenic purpura               | 100            | 66, 67            |
| Heparin-induced thrombocytopenia                  | 100            | 68                |
| Multiple sclerosis                                | 23-75          | 11, 83            |
| IgA nephropathy                                   | 32             | 75                |
| Diabetes mellitus                                 | 26-75          | 11, 16, 76, 77    |
| Hypoparathyroidism (autoimmune)                   | 100            | 11                |
| Acute pre-eclampsia                               | 50             | 78                |
| Rocky Mountain spotted fever                      | 50             | 79                |
| Viral infection                                   | Up to 18       | 11                |
| Borderline hypertension                           | None reported  | 80                |
| Hepatitis C virus mixed cryoglobulinemia          | 41             | 81                |

\* Reference numbers apply to references in the original full-length review from which this article is excerpted (Arth Rheum 2001; 44:1484-1494).

that the 130-kD membrane protein is an isoform of h-lamp-2, which differs primarily in its carbohydrate side chain.<sup>14</sup>

### SLE and primary APS

Recent analysis of AECA specificity in primary antiphospholipid syndrome (APS) and SLE revealed differences in both the pattern of antibody binding and band intensity between membrane antigens on HUVEC and human microvascular EC (HMEC).<sup>15</sup> In fact, of 17 primary APS sera, antibody binding to HUVEC membranes was found in 9 sera and to HMEC membranes in 7 sera. Binding at 72 to 79 kD was confined to HUVEC. Among 32 SLE sera, binding to HUVEC and HMEC membranes was detected in 17 and 22 sera, respectively, with binding at 135 to 155 kD being confined to HMEC. As mentioned, some anti-EC reactivity in APS may be directed to epitopes on phospholipid-binding proteins, especially  $\beta_2$ GPI.

### Takayasu's arteritis (TA)

In a previous study we were able to show entirely differential binding and activation of microvascular and macrovascular EC in various vasculitic conditions and diverse autoimmune disorders. With this regard, we obtained monoclonal anti-EC antibodies (mAbs) from a patient with TA.<sup>12</sup> Six mAbs were selected, the mixture of which produced 100% inhibition of binding of the original IgG from the patient with TA to HUVEC. All mAbs possessed high activity against macrovascular EC (ie, anti-HUVEC activity), but none had significant anti-microvascular EC (anti-human bone marrow EC:HBMEC) activity. Four of the 6 mAbs activated EC, which was manifested by increased IL-6 and von Willebrand factor secretion. The 4 mAbs induced EC expression of adhesion molecules and increased adhesion of monocytes to EC. In addition, these mAbs stimulated the nuclear translocation of the nuclear factor  $\kappa$ B transcription factor. Moreover, the immunohistochemistry studies demonstrat-

ed considerable anti-human-aortic EC activity of the mAbs, while anti-microvascular EC antibodies (from patients with heparin-induced thrombocytopenia) or normal human IgG did not react with human aorta. Again, the distinct predilection of the AECA mAbs to macrovascular antigens is compatible with the pathological characteristics of TA, which exclusively affects large arteries.

### Other diseases

*Behçet's disease* is preferentially a small-vessel disease, although large vessel involvement is observed in 15% to 35% of patients.<sup>16</sup> However, when the same patients' sera were exposed to microvascular (omental) or macrovascular (HUVEC) EC employing a cyto-ELISA, binding to microvascular EC was seen in 43% of patients' sera, whereas 26% of patients' sera recognized HUVEC.<sup>10</sup>

Progress on the isolation and culture of various EC has allowed comparison of biochemical and physiologic properties of EC from the micro- and macrovasculature. These cells share certain common features, including monolayer formation, production of factor VIII, and prostacyclin as well as the presence of Weibel-Palade bodies. EC from small capillaries, however, differ from the EC of large arteries and veins in their nutritional requirements and in their responses to growth and migration stimuli.<sup>17</sup> The antigenic heterogeneity of vascular endothelium was further elucidated employing immunocytochemical methods on different human vascular beds.<sup>18</sup> According to the results of this study, capillary EC strongly express MHC

class I and II, ICAM, and OKM5, which are variably weak to undetectable on large vessels. In contrast, large vessels strongly express von Willebrand factor and appear to constitutively express E-selectin. The authors anticipated that the capillary EC may be more efficient at antigen presentation or more susceptible to immune attack *in vivo*.

The concept of vascular bed-specific hemostasis and hypercoagulable states, recently presented by Rosenberg and Aird,<sup>19</sup> sheds additional light on phenotypic and functional differences between various EC. According to this concept, the endothelium integrates different extracellular signals and responds differently to the same endogenous (eg, local changes in blood flow) or exogenous injurious agents in different regions of the vascular tree.

### CONCLUSION

The differences between EC from large and small vessels and the consequent reactivity of AECA in small- and large-vessel diseases underscore the importance of using cells derived from vessels of appropriate size when studying macro- or microangiopathies. The wide range of AECA frequencies in a defined disease, as shown in Table 1, may be therefore attributed to the various sources of EC used for detection of AECA. Some sera apparently negative for AECA may be reactive if EC of appropriate types were employed. Consequently, it is rational to classify AECA into one of two groups of antibodies, against either microvascular or macrovascular EC.

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