



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

YEHUDA SHOENFELD, MD

Antibodies 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

From the Department of Medicine B, Center for Autoimmune Diseases, Sackler Faculty of Medicine, Tel-Aviv University, Israel. Address correspondence to Y.S., Center for Autoimmune Diseases and Department of Medicine B, Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel. E-mail: shoenfel@post.tau.ac.il

\* This article is excerpted, with permission, from a longer review of this topic published in *Arthritis & Rheumatism* (Praprotnik S, Blank M, Meroni PL, Rozman B, Eldor A, Shoenfeld Y. Classification of anti-endothelial cell antibodies into antibodies against microvascular and macrovascular endothelial cells: the pathogenic and diagnostic implications. *Arth Rheum* 2001; 44:1484-1494). *Arthritis & Rheumatism* © Copyright 2001 John Wiley & Sons.

**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.

### REFERENCES

- Lindquist KJ, Osterland CK. Human antibodies to vascular endothelium. *Clin Exp Immunol* 1971; 9:753-760.
- Tan EM, Pearson CM. Rheumatic disease sera reactive with capillaries in the mouse kidney. *Arth Rheum* 1972; 15:23-28.
- Meroni PL, Youinou P. Endothelial cell antibodies. In: Peter J, Shoenfeld Y, eds. *Autoantibodies*. Amsterdam: Elsevier Science BV; 1996: 245-248.
- Li SJ, Liu MF, Lei HY. Characterization of antiendothelial cell antibodies in the patient with systemic lupus erythematosus: a potential marker for disease activity. *Clin Immunol Immunopathol* 1996; 79:211-216.
- Del Papa N, Comfoti G, Gambini D, et al. Characterization of the endothelial surface proteins recognized by anti-endothelial antibodies in primary and secondary autoimmune vasculitis. *Clin Immunol Immunopathol* 1994; 70:211-216.
- Hill MB, Phipps JL, Cartwright RJ, Milford Ward A, Greaves M, Hughes P. Antibodies to membranes of endothelial cells and fibroblasts in scleroderma. *Clin Exp Immunol* 1996; 106:491-497.
- Hill MB, Phipps JL, Milford Ward A, Greaves M, Hughes P. Further characterization of anti-endothelial cell antibodies in systemic lupus erythematosus by controlled immunoblotting. *Br J Rheumatol* 1996; 35:1231-1238.
- Del Papa N, Gambini D, Meroni PL. Anti-endothelial antibodies and autoimmune diseases. *Clin Rev Allergy* 1994; 12:275-286.
- Meroni PL, D'Cruz D, Khamashta MA, Hughes GRV. Anti-endothelial cell antibodies: only for scientists or for clinicians too? *Clin Exp Immunol* 1996; 104:199-202.
- Cervera R, Navarro M, Lopez-Soto A, et al. Antibodies to endothelial cell in Behçet's disease: cell-binding heterogeneity and association with clinical activity. *Ann Rheum Dis* 1994; 53:265-267.
- Meroni PL. Endothelial cell antibodies (AECA): from a laboratory curiosity to another useful autoantibody. In: Shoenfeld Y, ed. *The Decade of Autoimmunity*. Amsterdam: Elsevier Science BV; 1999: 285-294.
- Blank M, Krause I, Goldkorn T, et al. Monoclonal anti-endothelial cell antibodies from a patient with Takayasu arteritis activate endothelial cells from large vessels. *Arth Rheum* 1999; 42:1421-1432.
- Kain R, Matsuyuki M, Exner M, et al. A novel class of autoantigens of anti-neutrophil cytoplasmic antibodies in necrotizing and crescentic glomerulonephritis: the lysosomal membrane glycoprotein h-lamp-2 in neutrophil granulocytes and a related membrane protein in glomerular endothelial cell. *J Exp Med* 1995; 181:585-597.
- Cacoub P, Ghillani P, Revelen R, et al. Anti-endothelial cell autoantibodies in hepatitis C virus mixed cryoglobulinemia. *J Hepatol* 1999; 31:598-603.
- Hill MB, Phipps JL, Hughes P, Greaves M. Anti-endothelial cell antibodies in primary antiphospholipid syndrome and SLE: pattern of reactivity with membrane antigens on microvascular and umbilical venous cell membranes. *Br J Haematol* 1998; 103:416-421.
- Schirmer M, Calamia KT, O'Duffy JD. Is there a place for large vessel disease in the diagnostic criteria of Behçet's disease? *J Rheumatol* 1999; 26:2511-2512.
- Zetter BR. The endothelial cells of large and small blood vessels. *Diabetes* 1981; 30(suppl 2):24-28.
- Page C, Rose M, Yacoub M, Pigott R. Antigenic heterogeneity of vascular endothelium. *Am J Pathol* 1992; 141:673-683.
- Rosenberg RD, Aird WC. Vascular-bed specific hemostasis and hypercoagulable states. *New Engl J Med* 1999; 340:1555-1564.