The Miscellany of Anti-Endothelial Cell Autoantibodies

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Endothelial cells lining the vasculature appear to be a target for immune-mediated assaut [1], conceivably through anti-endothelial cell antibodies. Originally detected by indirect immunofluorescence analysis with tissue sections as the substrate [2], the latter have since been sought using an enzyme-linked immunosorbent assay and are claimed to exist in most autoimmune states [3]. Among these are systemic lupus erythematosus and mixed connective tissue disease, as reported by Lage et al. in this issue of *IMAJ* [4]. These autoantibodies have aroused a great deal of controversy, raising uncertainty as to their practical usefulness. Can ELISA, still in use, detect all sorts of AECA? Moreover, can this test identify such or such vasculitis? Finally, are the AECA pathogenic and, hence, the reason underlying therapeutic depletion?

AECA are both heterogeneous and hard to identify. That is, the results obtained in a given pathology vary from one study to another. For convenience sake, unfixed human umbilical vein endothelial cells have long served as the substrate, notwithstanding the fact that AECA have been classified into antibodies against microvascular and macrovascular endothelial cells [5]. Because the number of HUVEC is restricted, which limits the test, a cell line, referred to as EA.hy926 cells, has been established by fusing HUVEC with permanent epithelial cells. The problem is that sera may contain antibodies to epithelial structures, as well as AECA. Yet, many techniques have been developed to assess the autoantibodies, including ELISA, radioimmunoassay, Western blotting and flow cytometry [6]. As highlighted by Adler et al. [7], there is a need for standardization.

Insights into the clinical relevance of AECA are just beginning to emerge. The increase in AECA titers precedes vasculitis flares in some patients [8]. What's more, elevated serum AECA levels parallel disease activity in patients with MCTD [4,9] and represent a potential marker for kidney involvement in those with SLE [10]. In fact, the target antigens change from one group of AECA to another, where membrane components, ligand-receptor complexes and so-called planted antigens have been incriminated. Unfortunately, the antigen status of endothelium from different sites is far from being completed, and indeed sera apparently negative to an EC type become positive if appropriate substrate cells are used. Cytokines can also render EC immunogenic, whereas similar resting cells are not. The diversity of AECA has thus frequently proven to be a stumbling block to the idea that they contribute to the diagnosis of vasculitis-associated diseases. A handful of antigens have, however, been identified, affording a new tool for the clinician in the management of inflammation. For example, the 60 kDa heat shock protein has been found in SLE [11], the ribosomal P protein Po in MCTD [12], and Toll-like receptor-associated beta-2 glycoprotein I in the antiphospholipid antibody syndrome [13]. As suggested by the finding that some of them cross-react with fibroblasts [14], it stands to reason that these results do not necessarily mean that AECA are specific for EC.

Next is the issue of the interpretation of this phenomenon. The presence of AECA in vasculitides does not imply causation, inasmuch as their production may follow rather than precede EC damage. Nonetheless, there is compelling evidence that they contribute to the disease, though no definitive demonstration supporting this hypothesis has emerged. The most persuasive argument has indeed come from the development of an idiotypic model of systemic vasculitis [15]. Various effects of AECA on the target cells have been described. EC can thus be activated, as first demonstrated by Del Papa et al. [16]. In their study, immunoglobulin G from three AECA-positive sera from patients with Wegener's granulomatosis enhanced the expression of adhesion molecules and led to the release of cytokines and chemokines. We have confirmed that a subgroup of AECA, but not all, activate the cells [17], while other AECA encourage the production of tissue factor, and thereby exaggerate coagulation [18].

Even worse, the binding of AECA from patients with systemic sclerosis induces EC into apoptosis [19]. As a result, anionic phospholipids are exposed, which may be important in triggering the production of antiphospholipid antibodies in connective tissue diseases. Lastly, it appears that some AECA recognize adenosine triphosphate synthase, the endogenous receptor for HSP60 [20], and thereby induces intracellular acidification.

ELISA = enzyme-linked immunosorbent assay
AECA = anti-endothelial cell antibodies
HUVEC = unfixed human umbilical vein endothelial cells

EC = endothelial cell
MCTD = mixed connective tissue disease
SLE = systemic lupus erythematosus

HSP60 = 60 kDa heat shock protein

To conclude, much uncertainty surrounds the significance of AECA. Studies are in progress to identify the cell surface epitope(s) recognized by apoptosis-causing AECA. Beyond any doubt, further work is warranted to illuminate their role in diseases, creating the potential for an autoamplifying loop of vascular damage.

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Capsule

Senescence surveillance of pre-malignant hepatocytes limits liver cancer development

Upon the aberrant activation of oncogenes, normal cells can enter the cellular senescence program, a state of stable cell-cycle arrest, which represents an important barrier against tumor development in vivo. Senescent cells communicate with their environment by secreting various cytokines and growth factors, and it was reported that this 'secretory phenotype' can have pro- as well as anti-tumorigenic effects. Kang et al. show that oncogene-induced senescence occurs in otherwise normal murine hepatocytes in vivo. Pre-malignant senescent hepatocytes secrete chemo- and cytokines and are subject to immune-mediated clearance (designated as senescence surveillance), which depends on an intact CD4+ T cell-mediated adaptive immune response. Impaired immune surveillance of pre-malignant senescent hepatocytes results in the development of murine hepatocellular carcinomas (HCCs), thus showing that senescence surveillance is important for tumor suppression in vivo. In accordance with these observations, ras-specific Th1 lymphocytes could be detected in mice in which oncogene-induced senescence had been triggered by hepatic expression of NrasG12V. The authors also found that CD4+ T cells require monocytes/macrophages to execute the clearance of senescent hepatocytes. The study indicates that senescence surveillance represents an important extrinsic component of the senescence anti-tumor barrier, and illustrates how the cellular senescence program is involved in tumor immune surveillance by mounting specific immune responses against antigens expressed in pre-malignant senescent cells.

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Eitan Israeli

"To know is to know that you know nothing. That is the meaning of true knowledge"

Confucius (551-479 BC), Chinese thinker and philosopher who emphasized personal and governmental morality, correctness of social relationships, justice and sincerity