MARKERS OF ENDOTHELIAL PERTURBATION AND DAMAGE

It is now well recognized that endothelial cell functions are altered in sites of acute and chronic inflammation, notably with respect to the attraction and signalling of leukocyte traffic into sites of inflammation by virtue of the expression of specific types of leukocyte adhesion molecule or leukocyte chemoattractant peptides [1, 2]. These processes are evident in disease conditions ranging from the inflamed synovium of the rheumatoid joint to endothelium overlying early atherosclerotic lesions in large blood vessels. Furthermore, there is good histological evidence of endothelial damage in autoimmune vasculitic and connective tissue diseases such as Wegener's granulomatosis and scleroderma.

The search for circulating markers that might indicate endothelial cell activation or damage, and thus could shed light on pathogenesis, or be useful reporters of disease activity or progress, has gone on for over a decade. Among the earliest choices was assay of angiotensin converting enzyme (ACE) activity in plasma, once it was realized that circulating ACE was predominantly derived from the endothelium, where it is an ectoenzyme. This has not proved to be very sensitive and suffers from the drawback of measuring enzyme activity, which can be altered by other interfering substances (not least clinically prescribed ACE inhibitors), rather than protein concentration.

von Willebrand factor (vWF) in plasma is secreted from endothelial cells. Although blood platelets also contain vWF, this makes a barely significant contribution, and therefore plasma vWF levels provide a selective marker of an endothelial cell function, with the substantial advantage over tissue-type plasminogen activator (tPA; also secreted by endothelium) of a long plasma half-life. There have now been many publications documenting significant increases in plasma vWF in populations of patients with a variety of inflammatory or vasculitic diseases e.g. [3, 4], with strong suggestions (though few hard data) that individual levels may increase predictively as clinical conditions worsen. Since vWF is easily measured by ELISA, it could thus provide a useful marker of altered endothelial function as a correlate of disease. There are, however, at least two drawbacks. One is practical—in addition to chronic elevation in disease states, vWF is transiently elevated during any acute phase response to infection in the same manner as CRP [5], and thus measurements (e.g. during acute infection) can be misleading. The second is theoretical—exactly how vWF secretion from endothelium is controlled so that circulating levels are chronically elevated is not well understood. For example it could be due to systemically or locally altered levels of cytokines or other mediators, but no obvious candidate has been identified. Increased vWF release could also be due to a superimposed level of frank endothelial damage from various causes, and therefore represent a measure of cell injury rather than cell activation: it would obviously be valuable to be able to distinguish between these possibilities.

The production of another classical mediator by stimulated endothelial cells, prostacyclin (PGI₂), can be measured accurately and non-invasively by determination of its major catabolite in urine. Early studies demonstrated that biosynthesis was increased in patients with atherosclerosis, probably as a consequence of enhanced platelet reactivity, shown by the concomitantly increased excretion of thromboxane (Tx) A₂ metabolites [6]. Subsequently comparable associated increases in PGI₂ and TXA₂ production have been found in patients with systemic sclerosis [7] and more recently in Henoch–Schönlein purpura, where a relationship between elevated production and disease activity was reported [8]. However, the analytical techniques needed to measure eicosanoid metabolites (e.g. high performance liquid chromatography) are not routinely available, which has undoubtedly limited the number of studies performed.

Over the last 5 years there has been an increasing recognition that autoantibodies recognizing endothelial cell antigens (AECA) are present in a proportion (often over 50%) of patients with autoimmune diseases with a vasculitic component [9]. Since AECA are in general clearly distinguishable from other diagnostic autoantibodies, such as anti-DNA, anti-cardiolipin or anti-ss, and are usually detected by ELISA using cultured endothelial cells as the target, there has been considerable interest in how AECA arise, what their antigenic targets may be, and whether they alter endothelial cell function themselves or are merely markers of altered structure and function. The answers to all these questions are, however, still at best equivocal; the cellular specificity of AECA is not absolute; and they are not present in all patients. Measuring AECA therefore cannot provide a generally applicable test as a marker of endothelial perturbation. Nonetheless, it is clear that further basic scientific enquiries concerning their specificity and activity, coupled with longitudinal studies to determine their relationship to disease activity or other clinical evidence of endothelial damage, will be valuable. It already seems apparent, for instance, that although mean AECA and vWF levels are both elevated in Wegener's disease, the levels of each in individual samples are not significantly correlated [10], suggesting that each marker is measuring something different about endothelial function.

In the last year antibody-based assays have been developed and are now commercially available to detect a series of circulating proteins that represent soluble versions of molecules normally present as integral plasma membrane components of endothelial cells. The first of these is thrombomodulin (Tm), an endothelial cell-specific glycoprotein that is an important regulator of activated thrombin, converting thrombin from a procoagulant to an anticoagulant by altering its substrate specificity so that it no longer cleaves fibrinogen but activates Protein C [11]. Soluble Tm levels were initially shown to be enhanced in patients with altered coagulant status, such as those on haemodialysis or with disseminated intravascular coagulation e.g. [12], but recent
reports have found elevated soluble Tm in patients with lupus, thrombotic thrombocytopenic purpura and other vasculitides, with correlations between increased levels and disease activity and indicators of prognostic interest [13, 14]. Interestingly, soluble Tm levels in patients with systemic sclerosis have been found to be in the normal range [C.R.M. Hay, personal communication]. It will clearly be of value to extend these early findings to studies of other rheumatoid diseases. Tm is, however, constitutively expressed by endothelial cells, and it is possible that assays for circulating forms of surface molecules that are only found when endothelial cells are activated, for example by inflammatory cytokines, could provide a more sensitive marker of disease progress. Soluble forms of several leukocyte adhesion molecules have now been detected, and of these E-selectin and the vascular cell adhesion molecule VCAM, which are both quite specifically expressed only on activated endothelium, e.g. in the small vessels of inflamed rheumatoid joint synovium [15–17], are prime candidates for further investigation. Assays for soluble intercellular cell adhesion molecule 1 (ICAM-1) are also available, though it must be remembered that this molecule is present on leukocytes and other cell types as well as endothelium. Preliminary data indicate that increased levels of each can be detected in patients where endothelial damage is expected, including those on haemodialysis and those with diabetes [18]. Most recently, it has been shown that plasma levels of soluble VCAM and ICAM-1 are both elevated in patients with RA whereas only soluble VCAM is raised in lupus [19, 20]. The elevation of VCAM, however (like vWF), was clearly associated with a concurrent acute phase response. Further studies are urgently needed to determine if measurement of soluble endothelial markers will provide more sensitive indicators of disease activity and endothelial cell injury in rheumatoid or vasculitic disease, and can give us greater insights into the mechanisms involved.

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REFERENCES