Soluble adhesion molecules in inflammatory and vascular diseases

J.C. Giddings

The Arthur Bloom Centre, Wales College of Medicine, Cardiff University, U.K.

Abstract
For many years the vascular endothelium was believed simply to provide a passive lining between circulating blood and extravascular tissue. It is now clear, however, that this monolayer of cells on the luminal surface of all blood vessels, provides a selective barrier that responds dynamically to various stimuli, and controls a complex series of cellular reactions and interactions. The current presentation describes the use of computer enhanced video recording to study interactions between endothelial cells and circulating blood cells, especially leucocytes. Subsequently, modern assays for soluble cell adhesion molecules and other cell receptors were assessed for potential use in routine clinical practice. The results demonstrated that adhesive mechanisms involving leucocytes and endothelial cells involve a range of interrelationships that cut across conventional views of haemostasis and leucocyte function. The findings also suggest that interplay between the vascular lumen and circulating blood cells might be vitally important in clinically demanding pathologies, such as life-threatening sepsis, ischaemic heart disease, atherosclerosis and cancer. The concepts provide challenging strategies for further investigation.

Introduction
Several lines of recent evidence have provided compelling results to support the concept that there is a close relationship between the mechanisms of inflammation, coagulation and fibrinolysis involving the vascular endothelium in vivo [1–14]. Diverse biological defence systems such as complement activation, kinin generation, regulation of the renin–angiotensin system, clearance of circulating lipids, leucocyte trafficking and haemostasis are now known to depend on an integrated series of intravascular events controlled by a relatively restricted number of common glycoproteins and signalling molecules. It is especially pertinent in this respect that endothelial cells, platelets and leucocytes express so-called CAMs (cell adhesion molecules) and other receptors that facilitate cell binding to the intact endothelium and to glycoproteins of the extracellular matrix. Disorderly synthesis or expression of these substances is evident under important clinical conditions, such as life-threatening infection, vasculitis, microangiopathy, neurological disease, atherosclerosis and thromboembolism. In addition, a number of inflammatory mediators such as lipopolysaccharide (endotoxin), tumour necrosis factor α and interleukin-1 together with the localized production of thrombin at sites of perturbed vascular endothelium induce a number of physiological responses that are probably pivotal in a range of pathological processes. The significance of interrelated mechanisms of this nature in clinical medicine is being highlighted by an ever-increasing literature that tends to cut across conventional disciplines and that could provide challenging strategies for future diagnosis and therapy. Therefore considerable attention has focused on the role of humoral immune mechanisms and the loss of endothelial integrity in the pathogenesis of cardiovascular disorders, especially those in which sepsis and systemic vasculitis appear to be major contributing features. We have attempted to examine these mechanisms in a number of human and animal experimental models.

Results and discussion
Using co-cultures of human endothelial cells and leucocytes under static conditions and under conditions representative of physiological blood flow, we have confirmed that the sequential events of leucocyte rolling and adhesion on the vascular monolayer, followed by extravasation, are governed by specific molecular interactions. In particular, we demonstrated that the initial rolling/adhesion mechanisms were depressed by monoclonal antibodies to P- and L-selectins and by annexin V. Firm adhesion was inhibited by anti-ICAM-1 (intercellular cell-adhesion molecule 1) and diapedesis was blocked by anti-CAM-1 (anti-PECAM). Furthermore, using an animal model of thrombogenesis in vivo [15], we demonstrated that non-overt endothelial perturbation mediated the incorporation of rolling leucocytes into a developing thrombus, potentially augmenting fibrinolysis [16].

We have also investigated the possible significance of CAMs in clinical medicine. Several methods have been proposed in recent years to evaluate endothelial disturbances in vivo, and assays have been devised to examine cellular responses to inflammation. The relative importance of tests of this nature has not been firmly established, however, and we are undertaking a range of studies designed to explore their use in routine clinical practice.

Key words: disseminated intravascular coagulation, endothelial cell, haemostasis, inflammation, leucocyte, soluble adhesion molecule, vasculitis.

Abbreviations used: CAM, cell adhesion molecule; ICAM-1, intercellular CAM-1; VCAM-1, vascular CAM-1; EK, disseminated intravascular coagulation; TF, tissue factor; TFPI, TF pathway inhibitor; TIM, thrombomodulin.

1 email giddings@cf.ac.uk

©2005 Biochemical Society
Initially, we evaluated the use of commercially available ELISAs for von Willebrand factor, TM (thrombomodulin) and CAMs (ICAM-1, VCAM-1, E-selectin and P-selectin, where VCAM-1 stands for vascular cell adhesion molecule-1) in the routine management of patients with vasculitis [8]. Patients were recruited over a period of approx. 12 months from regular hospital clinic visits. Venous blood samples were obtained at diagnosis or when symptoms of active disease were notably evident. Repeat samples were obtained at intervals over a period of up to 5 years during and after therapy. Normal control volunteers were also recruited over this time. The mean results of assays within the different patient groups demonstrated that in general, levels of circulating von Willebrand factor, TM and ICAM-1 were significantly increased. Similarly, the mean VCAM-1 and E-selectin assays were significantly raised in systemic lupus erythematosus, giant cell arteritis and glomerular nephritis. VCAM-1 tended to be high in systemic sclerosis. P-selectin tended to be significantly higher in some patients, especially in rheumatoid arthritis, although a very wide normal range was observed for this assay. There was a considerable overlap, however, between the assays on patients and those on normal controls and the measurements of different assays between groups correlated poorly with each other.

The levels of the different analytes tended to be higher in patients with the most severe clinical symptoms, and in a number of cases on repeated blood samples obtained after treatment, the levels decreased after effective therapy. The results were not straightforward, however, and did not consistently reflect disease activity. In many instances, assays were higher after treatment than before therapy. No correlation was observed between any of the ELISA results and other determinants of disease activity, such as erythrocyte sedimentation rate and C-reactive protein.

In a further study, we investigated especially the use of these assays with regard to overt DIC (disseminated intravascular coagulation) in intensive care medicine. Assays were performed on 63 surgical and trauma patients (29 DIC and 34 non-DIC) and compared with 20 healthy controls. In patients with DIC, there were significant increases in circulating P-selectin, E-selectin, ICAM-1 and VCAM-1 but not in L-selectin. There were also increases in soluble TF (tissue factor) and TM, but not TFPI (TF pathway inhibitor). The results appeared to reflect a probable link between adhesion molecule expression and TF activity that might be of significance in DIC, and that enhanced TF expression may not be adequately balanced by TFPI in these circumstances.

We have also utilized these assays to investigate the relationship between pre-existing disturbances in inflammatory and haemostasis markers and the risk of thrombotic and embolic events after cardiac surgery [17]. We conducted a longitudinal study to correlate haematological, biochemical and microbiological data with subsequent clinical endpoints in 375 consecutive patients undergoing heart valve replacement. The haematological data demonstrated that there were marked differences between patients, with many abnormal values preoperatively, not explicable by age-related variation or the presence of arterial disease. In addition, very high levels of inflammatory markers (tumour necrosis factor α and interleukin-6) were observed in a high proportion of patients, and the incidence of subsequent clinical complications appeared to correlate with evidence of pre-existing specific infection.

Overall, our results have highlighted the importance of interrelated mechanisms in the pathogenesis of haemostatic disorders. Measurements of soluble adhesion molecules in plasma might provide clues in general but appear not to be helpful for individual diagnostic purposes in a routine clinical setting. Sensitive analysis of the primary mechanisms of blood coagulation might be informative but preanalytical variables require careful attention.

These recent advances in knowledge of intercellular adhesive mechanisms have attracted considerable interest in the possibility of controlled pharmacological intervention for the treatment of various diseases [18,19]. For example, abrogation of neutrophil-mediated tissue damage without unduly compromising immune mechanisms could reduce morbidity in chronic disorders, such as parenchymal hepatic disease, rheumatoid arthritis, inflammatory bowel disease and Gram-negative sepsis. Similarly, strategies directed against other leucocyte and platelet interactions could provide novel methods for the treatment of high-risk patients with adult respiratory distress syndrome, myocardial ischaemia-reperfusion injury and thrombotic platelet disorders. In addition, specific adhesion antagonists may provide a means for useful therapy in certain malignancies, allergies and dermatological disorders.

At present such concepts are at an early stage of development but basic principles have been established and preliminary studies, mainly in tissue culture and in animal models, have been reported. However, there may be considerable restrictions on the use of site-directed anti-adhesion therapy. A number of apparently specific events are governed by several receptor–ligand mechanisms and inhibition of only one pathway may not necessarily be clinically effective. In contrast, a biologically significant overall reduction in leucocyte function by interfering with multiple adhesion pathways may involve intolerable side-effects. These and other considerations remain to be fully explored. Nevertheless, the development of innovative, effective and safe clinical therapies remains an attractive objective.

These studies have been supported by The British Heart Foundation, The Leukaemia Research Appeal for Wales and The Heart Research Fund for Wales. Cell culture and in vivo thrombogenesis studies were performed in collaboration with Professor J. Yamamoto and his colleagues, Faculty of Nutrition, Kobe Gakuin University, Japan.

References
9 Ishii, H. (1994) in Thrombomodulin and the Control of Haemostasis (Giddings, J.C., ed.), pp. 121–141, RG Landes, Austin, TX

Received 17 September 2004