REVIEWS

Vascular endothelium, cytokines, and the pathogenesis of inflammatory synovitis

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In the past 15 years, primarily as a direct consequence of the ability to isolate and culture vascular endothelial cells routinely, it has become obvious that the endothelium does not function simply as a selective permeability barrier. Endothelial cells are now known to contribute directly to the control of many aspects of vascular homeostasis, including the processes of blood coagulation and fibrinolysis, platelet activation, vasomotor tone and vascular permeability, vessel growth and remodelling, and leucocyte trafficking under normal and pathological conditions.¹

The most obvious changes in the inflamed rheumatoid joint that may reflect endothelial cell dysfunction include increased fluid and protein leakage into the tissue, coupled with leucocyte accumulation, and in the longer term, altered capillary density and anatomy in the hyperplastic synovium.² A recent review article in this journal discussed the possible role of hypoxic reperfusion injury in relation to endothelial cell pathology and joint damage.³ Here we review the recent advances in another area of endothelial cell biology—namely, the dramatic changes in endothelial cell function brought about by the action of specific cytokines or bacterial lipopolysaccharides, and how these changes may relate to pathogenesis in the joint, in particular by upregulating the ability of endothelial cells to support leucocyte traffic into the synovium.

Endothelial cell activation

Endothelial cells undergo morphological and functional changes at the sites of cell mediated immune and inflammatory reactions. These changes have been described as activation⁴ and can include proliferation, accumulation of intracellular organelles, hypertrophy, and increased matrix deposition. More recently, Pober⁵ defined endothelial cell activation as a quantitative change in the levels of expression of several specific gene products, which allows the cells to perform new functions. These include enhanced adhesion of specific leucocyte classes owing to the expression of surface glycoproteins, which is discussed in more detail below, and other alterations in cell function related to the control of vascular tone, platelet function, and blood coagulation.⁶

Leucocyte accumulation *in vivo*, in response to injection of supernatants from stimulated lymphocyte cultures, was first demonstrated by Dumonde *et al.*⁷ Enhanced lymphocyte adhesion to endothelial cells *in vitro* in response to such supernatants, known to contain lymphocyte

stimulatory molecules (lymphokines), was first reported by De Bono.⁸ Purified proteins from these mixtures, now known as cytokines since they act on many cell types other than lymphocytes, have subsequently been shown to act singly or in combination on endothelial cells to upregulate leucocyte adhesion. Thus interleukin 1 enhances neutrophil and lymphocyte adhesion with different time courses, whereas interferon γ enhances only lymphocyte adhesion.⁹⁻¹¹

Neutrophil-endothelial cell adhesion mechanisms

The initial recruitment of neutrophils to an inflammatory site is primarily dependent on chemotactic factors generated in the tissue, such as the activated complement fragment C5a, leukotriene B₄, or platelet activating factor. Neutrophils or monocytes stimulated by chemotactic agents, whether generated exogenously or presented by the endothelial cells, transiently show enhanced adhesion to endothelium.¹²⁻¹⁴ Longer term regulation of neutrophil adhesion to endothelial cells, particularly over periods of hours rather than minutes, is brought about by the induction of endothelial protein synthesis in response to cytokines.

ELAM-1 has recently been identified and characterised as an inducible surface glycoprotein that is important for neutrophil adhesion. Basal expression is undetectable, but when endothelial cells are exposed *in vitro* to interleukin 1, tumour necrosis factor, bacterial lipopolysaccharide, or lymphotoxin ELAM-1 expression is found after one to two hours, peaks at about six hours, and declines towards zero by 24 hours.^{10 15 16} Experiments with a blocking monoclonal antibody to ELAM-1 confirmed that the increased neutrophil adhesion, which is temporally correlated with ELAM-1 expression, was significantly inhibited,¹⁷ and transfection of ELAM-1 cDNA into COS cells showed that expression of this molecule at the cell surface was sufficient to induce neutrophil adhesion.¹⁸

ELAM-1 is also expressed on endothelial cells *in vivo* in response to the induction of delayed hypersensitivity reactions,^{19 20} and in skin explant cultures after stimulation of mast cell degranulation, where the activator appears to be tumour necrosis factor.²¹ Interestingly, the *in vivo* expression of ELAM-1 was more prolonged (still strongly positive at 24 hours) than occurs *in vitro*. The presence of ELAM-1 on endothelium in rheumatoid synovium has been reported in a recent abstract²²; and in preliminary experiments we detected ELAM-1 by immunofluorescence on a proportion of endothelial cells

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in synovial samples from patients with rheumatoid arthritis but not from those with osteoarthritis (Kaul A, unpublished observations).

On the neutrophil a member of the integrin family of adhesive ligands, CD11b/CD18, is essential for neutrophil adhesion and emigration in vitro and in vivo.²³ CD11b/CD18 does not interact with ELAM-1, but with intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 molecules present on non-activated endothelial cells.^{24 25} ICAM-1 expression increases on cytokine activated endothelium, but with a slower time course that more closely correlates with enhanced lymphocyte adhesion (see below). As yet, the neutrophil receptor for ELAM-1 has not been identified. In addition, Dobrina *et al* have provided evidence that part of the adhesion of neutrophils to stimulated endothelium is independent of ELAM-1,²⁶ suggesting that other molecules are involved. One candidate is GPI140: this molecule, rapidly translocated to the surface of endothelial cells in response to thrombin or histamine, where it transiently upregulates neutrophil adhesion, has also been implicated recently in adhesion to endothelial cells stimulated by lipopolysaccharide.^{27 28}

Further long term regulation of neutrophil recruitment to inflammatory sites may occur as a result of cytokine induced synthesis and secretion of other proteins by endothelium. These include colony stimulating factors that can act on bone marrow to accelerate myeloid cell production and release.²⁹ In addition, endothelial cells stimulated by lipopolysaccharide or cytokine release interleukin 8 related molecules, which are potent chemotactic agents for neutrophils and monocytes.³⁰ Gimbrone *et al* have recently identified as interleukin 8 an endothelial mediator that downregulates neutrophil adhesion to activated endothelial cells.³¹ This is likely to be an example of a phenomenon common to all neutrophil chemotactic agents, whereby long term exposure desensitises neutrophil responsiveness, and it may be relevant in vivo to the temporal control of neutrophil emigration.

Lymphocyte-endothelial cell adhesion mechanisms

Normal lymphocyte traffic—that is, recirculation between the bloodstream and lymphoid tissues, occurs by specific adhesive interactions between specialised postcapillary venules, lined by large columnar endothelial cells that are histochemically distinct from the flattened endothelial cells elsewhere.³² Unlike endothelial cells in most normal vessels these 'high' endothelial cells (HEC) express readily detectable levels of class II major histocompatibility complex (MHC) antigens.³³ Morphologically similar HEC develop in chronic inflammatory lesions in a variety of diseases, including rheumatoid arthritis, Sjögren's syndrome, and within tumours, where lymphocyte infiltration is evident.^{34–36} These studies, together with the sparseness of HEC in athymic mice,³⁷ indicate that the morphological changes are a consequence of the secretion of lymphocyte products, which perhaps in turn lead to phenotypic alterations in endothelial function that enhance lymphocyte accumulation.

Normal lymphocyte subclasses vary in their ability to bind to HEC from different lymphoid organs. Thus B cells bind preferentially to HEC in gut associated lymphoid tissue, where T cells bind preferentially to peripheral lymph node HEC.³⁸ Furthermore, some lymphoma cell lines bind almost exclusively to HEC in one or the other tissue.³⁹ This suggests that lymphocytes and HEC express organ specific receptor-ligand pairs to regulate the homing patterns observed. In humans, homing receptors on the lymphocyte surface are defined by the Hermes series of monoclonal antibodies.⁴⁰ These bind to 'addressin' molecules on HEC. Several addressins have been defined in mouse and man and shown to be induced by interferon γ .^{41 42} The human addressin identified by monoclonal antibody HECA-452 is found in organised lymphoid tissue and at certain chronic inflammatory sites, including synovial HEC.⁴³ Some antibodies that block lymphocyte binding to HEC in lymphoid tissue do not inhibit adhesion to HEC in inflamed synovium.⁴⁴ It therefore remains an open question whether as yet undefined addressins are expressed in rheumatoid synovium. A selective adhesion mechanism is suggested by the greater ability of some lymphocytes with a preference for gut associated normal lymphoid tissue to adhere to HEC in inflamed synovium. This is also consistent with the clinical link between inflammatory bowel disease and synovitis, and the possibility of shared antigenic stimuli—for example, yersinial or mycobacterial products.⁴⁵ Nonetheless, a major influence on lymphocyte infiltration and accumulation in the inflamed joint is likely to be the local expression of tissue non-selective endothelial cell surface adhesive molecules in response to cytokines, as discussed below.

In vitro experiments suggest that the induction of increased lymphocyte adhesion to endothelium in response to cytokines is slower and longer lasting than for neutrophils, with a maximum after about 24 hours.⁶ This correlates well with increased levels of ICAM-1 expression on endothelial cells, which peak at >10-fold higher than basal levels.⁴⁶ ICAM-1 and ICAM-2 are each ligands for the lymphocyte adhesion molecule CD11a/CD18 (lymphocyte function associated antigen-1 (LFA-1)).²⁴ Increased endothelial ICAM-1 expression is also found in vivo at sites of delayed hypersensitivity reactions or after tumour necrosis factor and interferon γ injection,^{20 47} and its physiological expression on endothelium is more intense in T cell dependent areas of lymph nodes.⁴⁸ In addition to the cytokines mentioned previously, interferon γ enhances lymphocyte adhesion and ICAM-1 expression in vitro, though it does not upregulate neutrophil adhesion.^{9 48} Uniquely, interferon γ also induces the expression of MHC class II molecules on the surface of endothelial cells in vitro,^{49 50} which, as noted above, are present on HEC.

Blocking antibodies to CD11a/CD18 significantly inhibit the adhesion of lymphocytes to unstimulated endothelial cells but only weakly inhibit lymphocyte adhesion to activated endothelial cells.⁵¹ Also, patients in whom all leucocytes lack CD11/CD18 show severely impaired

neutrophil adhesion and emigration but apparently normal lymphocyte recruitment to inflammatory sites.²⁵⁻⁵² Thus mechanisms independent of ICAM-1 must play a part in lymphocyte adhesion and emigration. Two studies have indicated that MHC class II molecules may be involved.⁵³⁻⁵⁴ In addition, the surface glycoprotein VCAM-1 (vascular cell adhesion molecule-1) has been implicated as one such molecule,⁵⁵ and it may be involved in the ICAM-1 independent stimulated adhesion of T cells found when endothelial cells are exposed to interleukin 4.⁵⁶ The lymphocyte receptor for VCAM-1 is now known to be the integrin VLA-4,⁵⁷ and antibodies to either VLA-4 or VCAM-1 reduce lymphocyte adhesion to endothelial cells.⁵⁸

The discovery that endothelial cells express MHC class II antigens in response to interferon γ led to the finding that endothelial cells activated in this way in vitro efficiently present antigen to primed T cells and cause T cell proliferation.⁵⁹⁻⁶⁰ The mechanisms by which this occurs might include the stimulated synthesis and secretion of interleukin 6 by endothelium⁶¹ (which helps T cells to secrete interleukin 2, a lymphocyte mitogen), though smooth muscle cells and fibroblasts can also be stimulated by interferon γ to express class II molecules and release interleukin 6, but they do not support lymphocyte proliferation.⁶² Recently, an additional endothelial cell-specific mechanism, requiring cell contact between lymphocytes and endothelium and involving lymphocyte CD2 surface molecules, has been described.⁶³

Cytokines and rheumatoid arthritis

Until recently, evidence about the presence of cytokines in synovial tissue or fluid has been confusing, with variable results depending on the type of assay used. The situation has become somewhat clearer with the realisation that inhibitors of the activity of cytokines may be concomitantly present in vivo, though they may not be locally generated as synovial cultures synthesise substantial amounts of several cytokines.⁶⁴ Thus the presence of interleukin 1 in synovial fluid from patients with rheumatoid arthritis is more easily detectable by immunoassays than bioassays,⁶⁵ but interleukin 1 activity is found in culture supernatants from rheumatoid synovium⁶⁶ and interleukin 1 mRNA is present in synovial tissue.⁶⁷ Interestingly, in cultured synovium interleukin 1 concentrations correlated well with the extent of vascularity and with MHC class II antigen expression on synoviocytes and endothelium.⁶⁶ Tumour necrosis factor protein has also been detected in synovial tissue and fluid from rheumatoid patients.⁶⁴ Interleukin 6, which is abundantly produced by endothelial cells activated in vitro,⁶¹ is also present in excess in rheumatoid synovial fluid.⁶⁸

In contrast, surprisingly little interferon γ is found in synovial fluid or produced by explants of rheumatoid synovium,⁶⁹ despite the presence of class II antigens, whose induction is presumed to be due to interferon γ . This lack of interferon γ correlated with a relative lack of other

detectable T cell derived cytokines (interleukins 2 and 3),⁷⁰⁻⁷¹ and perhaps implies that T cells in the inflamed synovium are in some way inhibited from synthesising cytokines.

Conclusion

The experimental evidence summarised above indicates that cytokines activate endothelial cells in ways that lead to leucocyte accumulation and emigration into tissues. Thus although endothelium has not yet been ascribed a role in the initiation of inflammatory synovitis, it is likely to be intimately involved in the regulation of disease progression and pathogenesis, and particularly to cooperate in the process of leucocyte recruitment. Further work is urgently needed to define more closely the presence and consequences of activated endothelium in rheumatoid synovium. The rapidly emerging power of molecular biological techniques to detect and localise the sites of synthesis and action of cytokines will contribute greatly to this. A further valuable aid will be the availability of microvascular endothelial cells from rheumatoid synovium, the successful culture of which has very recently been reported,⁷² and we have found the technique to be reproducible. Although we feel that insufficient thought has been given as yet to designing in vitro experiments under conditions that more closely mimic the environment of the diseased synovium—that is, hypoxia and acidosis,³ we are confident that significant progress in this area, linking the advances in our understanding of basic endothelial cell biology to the elucidation of the pathogenesis of rheumatoid synovium, will be made in the near future.

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