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Endothelium and regulatory cell interactions

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Abstract
Endothelial cells are injury targets in vasculitis and other diseases. However, their abilities to regulate their own fate are becoming increasingly recognized and may influence their susceptibility to injury in different vascular beds.

Introduction

Endothelial cells sit at the interface between the circulating blood or lymphatic fluids and tissues. They are well placed to select white blood cells from the circulation for capture and transit into tissues. For example, lymphocytes may be selected during physiological recirculation across lymphatic endothelium, or various leukocyte subtypes may be captured by vascular endothelium within an inflammatory focus after the endothelium has developed an 'activated' surface phenotype in response to pro-inflammatory molecules. Thus, the selective properties of vascular endothelial cells for circulating leukocytes may be influenced by tissue-restricted expression of selection molecules and/or by the response of the endothelium to local mediators of the inflammatory response. When interactions occur between endothelium and leukocytes, there is the potential for functional responses of either the endothelial cell or the leukocyte to be altered. Interactions between endothelial cells and the smallest blood element, the platelet, are usually indicative of endothelial injury but the molecular events underlying this are still highly regulated.

Of course, interactions may also occur between the endothelial cell and other cells of the vascular wall or nearby tissues that may regulate the functions of the endothelial cell. The pericyte is receiving increasing attention in this regard.

Heterogeneity of vascular endothelium

It is important to remember from the outset that endothelial cells are heterogeneous in structure (Table 1) (1), as well as presenting phenotypically distinct surface molecular profiles. Heterogeneity is linked to function, which is well illustrated by the glomerular endothelium that is the only endothelium to have fenestrations without diaphragms, presumably to facilitate ultrafiltration by allowing direct access to the underlying basement membrane. Sinusoidal endothelial cells are present in spleen, bone marrow and liver where they lack tight junctions and sit on a scanty basement membrane. Hepatic sinusoidal endothelial cells are discontinuous with interspersed kupffer cells and open fenestrations arranged in sieve plates. In lymph nodes, high endothelial venules contain morphologically distinct endothelial cells that select naive T cells for entry to the node, while phenotypically distinct endothelial cells are present in lymphatic vessels.

Leukocyte interactions with the vascular endothelium

The three major leukocyte types found in blood, namely neutrophils, monocytes and lymphocytes, can all adhere to classical vascular endothelium but their physiological roles and fates differ after transmigration. They are usually attracted to sites of antigen challenge such as microbial invasion, transplanted allogenic tissues, or vaccine deposits, participating there in an inflammatory response. Endothelial cell adhesion molecules and their complementary ligands on leucocytes, act in concert with chemokines and their receptors to promote the leukocyte recruitment, comprising leukocyte rolling over the endothelial surface, firm adhesion and transmigration; the molecular interactions underlying these interactions are well delineated and have been reviewed elsewhere (2). Variable degrees of stimulus specific and tissue specific responses may fine-tune the outcome. The processes controlling the extent of these responses and that lead to termination or resolution of the leukocyte migratory response are also beginning to be elucidated. The annexin-1 system is a good example of the active control of the resolution phase. Neutrophils have high cytoplasmic levels of annexin 1, which are augmented in response to glucocorticoids and cytokines. During the early phase of the inflammatory response when neutrophils are adherent

Table I. Properties of endothelial cells from different vascular beds.

Tissue	Type of endothelium	Diaphragm	Basement membrane	*PV-1 present
Peritubular capillaries	Fenestrated	Yes	Yes	Yes
Endocrine pancreas	Fenestrated	Yes	Yes	-
Exocrine pancreas	Fenestrated	Yes	Yes	Yes
Adrenal cortex	Fenestrated	Yes	Yes	Yes
Intestinal villi	Fenestrated	Yes	Yes	Yes
Eye choriocapillaries	Fenestrated	Yes	Yes	-
Brain choriocapillaries	Fenestrated	Yes	Yes	-
Glomerular capillaries	Fenestrated	No	Yes	No
Hepatic sinusoidal	Fenestrated	No	No	-
Skeletal muscle	Continuous	-	Yes	No
Aorta	Continuous	-	Yes	No
Coronary	Continuous	-	Yes	No
Lung alveolar	Continuous	-	Yes	-
Spleen	Discontinuous	No	Not exposed	-
Bone marrow	Discontinuous	No	Not exposed	-

*PV-1 is an antigen expressed by fenestrated endothelial cells.

to endothelium, annexin-1 translocates to the plasma membrane and interacts with specific G-protein coupled receptors to control the extent and rate of neutrophil migration (3). Absence of annexin-1 augments neutrophil recruitment and extent of activation.

Endothelial cells as regulators of an anti-inflammatory state

A number of diverse observations point to the endothelium as playing important roles in regulation of unwanted inflammatory or immune responses.

Neutrophil granulocytes are key cells of the innate immune system, leaving the blood to invade tissues where they phagocytose micro-organisms and tissue debris; on activation they undergo a respiratory burst, releasing reactive oxygen species and granule contents that include myeloperoxidase, serine proteases (proteinase 3, elastase) and metalloproteinases. The release is both external to the cell and into endocytic vacuoles for destruction of phagocytosed bacteria. In the vasculitis arena, it has been supposed that ANCA induce dysregulated activation of neutrophils while adherent to the endothelium, with superoxide release or degranulation focusing injury onto the endothelial cell. We believe this view is an oversimplification since, when neutrophils are co-cultured with endothelium, an ANCA-stimulated neutrophil respiratory burst is markedly downregulated by adenosine that is produced by endothelial cells (4). Adenosine binds to specific receptors on the neutrophil and inhibits the respiratory burst. This is a generic mechanism that operates with other forms of neutrophil activation also, offering the endothelium a degree of protection during neutrophil recruitment. In contrast, the ANCA-induced degranulation response is not inhibited by adenosine, so that serine proteases are still available to cause proteolytic damage to adjacent cell surface proteins.

Whilst endothelial cells can act as antigen-presenting cells for conventional cytotoxic MHC class-I restricted CD8 T lymphocytes, their abilities to induce proliferation of CD4 T lymphocytes are poor. Recently, studies have demonstrated that alloantigen presentation to CD4 T lymphocytes activates and induces $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells. These regulatory cells are capable of inhibiting proliferation of alloreactive T lymphocytes both *in vitro* and *in vivo* (5). In general it is easier to demonstrate these types of interactions with allogeneic interactions where precursor numbers of cells capable of direct allorecognition are high. In autoimmune settings, numbers of cells capable of recognition of endothelial autoantigens are likely to be low, but the principles affecting interactions may still apply.

Endothelial cells may also encourage development of cytotoxic CD8 T lymphocytes (CTLs) with unusual properties. In experimental systems, purified CD8 T lymphocytes that have been cultured with allogeneic endothelial cells and exogenous IL-2, can differentiate into allospecific, MHC class I-restricted cytotoxic lymphocytes, some of which may be endothelial cell-selective CTL. Such endothelial cell–stimulated CTL have a stable phenotype in the absence of continued stimulation by endothelium and, importantly, are poor secretors of the cytokines TNF and interferon-γ, which are believed to be key drivers of chronic allograft vasculopathies (6). Thus, intact endothelial cells may actually suppress the profibrotic activity of proinflammatory cytokines within the subendothelial microenvironment (7). Endothelial cells also profoundly suppress CD8 T cell activation by professional antigen-presenting cells.

Provision of regulation by complex interactions between endothelium and cellular components

Microvascular cell-cell interactions are important in generation of regulatory molecules. This is well illustrated by lipoxygenase-derived eicosanoids such as the leukotrienes and lipoxins (8). Leukotrienes are proinflammatory compounds formed via the 5-lipoxygenase pathway and are potent mediators of neutrophil recruitment and activation. Leukocytes are the principle cell types expressing 5-lipoxygenase and hence for leukotriene production. However, important amplification of leukotriene production may occur via transcellular biosynthetic pathways whereby leukotrienes released from leukocytes may be transiently taken up by endothelial cells and further transformed into bioactive products.

In contrast to leukotrienes, lipoxins are 'braking signals' for neutrophil recruitment. Here too, transcellular biosynthetic pathways co-operate for their formation. Leukotrienes produced by neutrophils may be taken up platelets and converted via 12-lipoxygenease into anti-inflammatory lipoxins. Interestingly, lipoxins may effect inhibition of neutrophil functions by binding to similar receptors as does annexin-1 (see above). The anti-inflammatory effects of lipoxins are underscored by

the effects of aspirin which blocks endothelial cyclooxygenase-2 enzymes; this diverts arachidonate metabolism into formation of novel lipoxygenase-derived eicosanoids. Thus, in the presence of aspirin, endothelial cells can synthesise 15-(R)-HETE that, following transcellular uptake by neutrophils, is converted to anti-inflammatory lipoxin epimers.

Wider roles of endothelium in regulation

In vasculitis, endothelial cells are damaged and increased numbers of endothelial cells with an inflammatory phenotype are present within the circulation (9). This would suggest that there is an increased need for endothelial cell renewal in vasculitis. However, the circulating endothelial cells with inflammatory phenotype can inhibit growth of those endothelial cells that have the phenotype of precursor cells. This points to major disturbances in regulation of precursor endothelial cell generation.

Whether vasculitis is associated with more fundamental disorders of human stem cell development is not known at present. However, this seems possible given that sinusoidal endothelial cells in bone marrow may contribute to one of the two architecturally distinct niches that support human stem cell development and this may be threatened by both the underlying vasculitic disease process and by the use of immune suppressing drugs (10).

Summary

Endothelial cells may be targets for injury in vasculitis. However, increasingly the regulatory roles of this multi-functional cell are becoming recognized.

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