Cytokine-Endothelial Interactions in Inflammation, Immunity, and Vascular Injury

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ABSTRACT
This paper reviews the evidence that cytokines induce a variety of functional and structural alterations in endothelium and that cytokine-endothelial interactions play important roles in the evolution of inflammatory and immune responses. The effect of cytokines, particularly interleukin-1 and tumor necrosis factor, on leukocyte-endothelial adhesion has led to the discovery of several endothelial adhesion molecules, and the molecular and biological characteristics of these are described. Finally, the review discusses the possible contribution of cytokine-induced activation to vascular injury in such pathological processes as septic shock, the Shwartzman reaction, delayed hypersensitivity, and immune-mediated vasculitis.

Key Words: Endothelial activation, cytokines, inflammation, vascular injury

Cytokines are a heterogeneous group of polypeptides which are most commonly elicited as part of the immune response to antigen and which mediate various aspects of inflammation and immunity (1). Most of the earlier work on cytokines was performed in leukocyte cultures from blood or lymphoid tissues, leading to the conclusion that the leukocytes were the principal targets of cytokine actions and leading to the use of the term interleukins. It is now clear, however, that other tissue cells are important and are often major targets of cytokine action. Among these tissues is vascular endothelium, and a large body of work suggests that cytokines induce in endothelial cells a variety of structural and functional alterations (often called endothelial "activation") and that cytokine-endothelial interactions may play important roles in inflammation, immunity, and vascular injury. This mini-review will focus on those interactions which may be particularly relevant to inflammatory-immune responses in the kidney and on evidence that such cytokine effects on endothelium may play a role in vascular injury in vivo. The cytokines discussed will be limited to interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and gamma interferon (IFN-γ), since these are the cytokines that appear to have the most profound endothelial effects. A more detailed account of other endothelial-cytokine interactions has been published elsewhere (1).

THE EFFECTS OF CYTOKINES ON LEUKOCYTE-ENDOTHELIAL ADHESION

One of the earliest, and possibly most important, events in inflammation is the adhesion of leukocytes to the endothelium of postcapillary venules as a prelude to their emigration across the vascular wall. Lymphocyte adhesion to endothelium is also one of the earliest events in the delayed hypersensitivity reaction, and monocyte attachment to arterial endothelium is a prominent feature of early experimental atherosclerosis. Both leukocytes and endothelium contribute to the increased adhesion. One of the major, and best studied, actions of cytokines on endothelium is their effects on endothelial adhesivity. IL-1 and TNF stimulate increased adhesivity of endothelial cells in culture for polymorphonuclear leukocytes, eosinophils, basophils, monocytes, and, in certain endothelia, lymphocytes (2-10). For neutrophils, stimulated adhesion is maximal at 4 to 6 h and then declines but remains at above control values at 24 h. In the case of certain leukocyte cell lines (for example, HL-60), adhesivity declines to baseline values by 12 h (11). T lymphocytes show elevated basal binding that increases after 4 h and then declines to basal levels by 24 h (6). These effects of IL-1 and TNF are blocked by RNA and protein synthesis inhibitors (11) and are mediated by the induction and cell surface expression of endothelial cell adhesion molecules that serve as ligands for complementary molecules on leukocytes. With the use of monoclonal antibodies and molecular techniques, several candidate molecules have been identified and shown to account for this increased adhesion.
Endothelial-Leukocyte Adhesion Molecule-1 (ELAM-1)

This molecule, initially identified by monoclonal antibodies H4/18 and H18/7, is a 115-kDa single chain glycoprotein which is induced on the endothelium in vitro at the height of increased adhesion of neutrophils and HL-60 cells (4 to 6 h). Antibodies to ELAM-1 markedly inhibit adhesion of these cell types (3). The structure of ELAM-1 has recently been determined by cDNA cloning (12). ELAM-1 belongs to a family of adhesion proteins characterized by a lectin-like domain at the amino acid terminus, a domain bearing homology to epidermal growth factor, and a tandem array of domains harboring consensus repeats similar to those occurring in certain complement regulatory proteins. The other two proteins of this family (called "selectins" by Bevilacqua) are also adhesion molecules that may play a role in leukocyte-endothelial interactions. The first is granule membrane protein 140 (GMP-140) (13) (also called PADGEM, for platelet activation-dependent granule exocytosis marker) (14), a protein which is normally present on the membrane of α-granules of platelets and the Weibel-Palade bodies of endothelium but which rapidly redistributes to the surface of these cells upon stimulation with thrombin and histamine (15). GMP-140 has been recently implicated in the adhesion of neutrophils to endothelium (16) and neutrophils to platelets (14). The second adhesion protein of the same family is MEL-14, a glycoprotein present on lymphocytes which serves as a homing receptor to high endothelial venules of lymphoid organs (17). The human homolog of MEL-14 has recently been cloned and recognized as Leu-8, which also has the characteristic structure of the selectin family of adhesion proteins (18). It is to be emphasized that IL-1 and TNF, which induce ELAM-1, do not stimulate either GMP-140 or MEL-14 surface expression. The neutrophil receptor that binds to ELAM-1 is currently unknown.

Intercellular Adhesion Molecule-1 (ICAM-1)

ICAM-1 is an 80- to 90-kDa single chain glycoprotein which is normally present on endothelial cells, lymphocytes, and fibroblasts, but whose surface expression is markedly increased from basal levels by treatment with IL-1 and TNF-α (19,20). ICAM-1 stimulation differs from ELAM-1 stimulation in that increased expression is slow, plateauing at 24 h, and ICAM-1 expression is sustained as long as the cytokine remains in the culture medium. Antibody-blocking experiments in vitro implicate ICAM-1 as an adhesion molecule for neutrophils (21) and B lymphocytes (19) in cytokine-stimulated endothelia, but not, apparently, for T lymphocytes. However, both neutrophils and T-lymphocytes utilize ICAM-1 to transmigrate across cultured endothelial monolayers. The receptor on leukocytes that interacts with ICAM-1 is lymphocyte function-associated antigen (LFA-1), also known as CD-11a/CD18 (19). A second endothelial cell ligand for LFA-1, called ICAM-2 (22), is not subject to stimulation by IL-1 and TNF. It should be stressed that increased expression of ICAM-1 can also be induced by IFN-γ and on other cell types (such as dermal fibroblasts, lymphocytes, and epithelial cells), whereas ELAM-1 expression is not induced by IFN-γ and is endothelium specific. Structurally, ICAM-1 and ICAM-2 are members of the immunoglobulin gene superfamily (22, 23).

INCAM-110 or VCAM-1

Recently, several laboratories reported the identification of a 110-kDa IL-1-inducible endothelial molecule which appears to mediate binding of monocytes and lymphocytes. Rice and Bevilacqua (24) initially discovered this molecule in studies involving adhesion of certain melanoma cell lines to IL-1-induced endothelium. They raised a monoclonal antibody to IL-1-treated endothelial cells, named E1/6, which inhibited IL-1-stimulated melanoma cell adhesion. They found that this antibody also blocked the adhesion of lymphocytes and monocytes (25). The molecule is present at basal levels in normal endothelium but is markedly stimulated by IL-1 and TNF, with peak expression at about 24 h, coinciding with the peak of increased adhesion of the melanoma cell lines (24). The molecule, designated inducible cell adhesion molecule-110 (INCAM-110) by Rice et al. is also present on other cell types, including follicular dendritic cells of lymph node and certain epithelia, and its expression is markedly stimulated in vivo in certain immune reactions, such as delayed hypersensitivity (25). Osborn et al. (26), by using DNA subtraction techniques, cloned an IL-1-inducible endothelial cell molecule which also mediated the adhesion of lymphocytes and monocytes and which they designated vascular cell adhesion molecule-1 (VCAM-1). An antibody with similar characteristics as E1/6 reacts with VCAM-1 (27). Structurally, VCAM-1 is a member of the immunoglobulin gene superfamily and interacts with the leukocyte-integrin VLA-4 (28). More recent studies suggest that INCAM-110 and VCAM-1 are the same molecule (Bevilacqua and Arrufo, unpublished data).

Table 1 summarizes the currently known adhesive interactions between endothelium and neutrophils and lymphocytes. Other IL-1/TNF-inducible leukocyte-adhesion molecules almost certainly exist, and undoubtedly future studies will further expand the list of ELAMs. It must be emphasized, however, that all of these interactions have been derived from studies in culture, and the question remains as to which...
TABLE 1. Endothelial adhesion molecules

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Structure</th>
<th>Normal Localization</th>
<th>Stimulated Localization in Endothelium</th>
<th>Ligand on Leukocyte</th>
<th>Possible Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMP-140</td>
<td>Selectin</td>
<td>Platelet α-granule; Endothelial Weibel-Palaide body</td>
<td>Redistributed to surface by histamine, thrombin</td>
<td>Unknown</td>
<td>Rapid neutrophil adhesion</td>
</tr>
<tr>
<td>ELAM-1</td>
<td>Selectin</td>
<td>Not present</td>
<td>TNF/IL-1 induced; Endothelial selective</td>
<td>Unknown</td>
<td>Early (4 to 6 h) neutrophil adhesion</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Immunoglobulin</td>
<td>Endothelium, fibroblasts, lymphocytes</td>
<td>Upregulated by TNF, IL-1 IFN-γ</td>
<td>LFA-1</td>
<td>Neutrophil and lymphocyte adhesion</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>Immunoglobulin</td>
<td>Endothelium, fibroblasts, lymphocytes</td>
<td>Not upregulated by cytokines</td>
<td>LFA-1</td>
<td>Lymphocyte adhesion</td>
</tr>
<tr>
<td>INCAM-110/ VCAM-1</td>
<td>Immunoglobulin</td>
<td>Endothelium, follicular dendritic cells, certain epithelia</td>
<td>Upregulated by TNF, IL-1</td>
<td>VLA-4</td>
<td>Lymphocyte and monocyte adhesion</td>
</tr>
</tbody>
</table>

of these adhesion molecules plays a role in in vivo. Although IL-1 and TNF induce leukocyte accumulation when injected intradermally in a variety of experimental animal models, the relationship of these adhesion molecules to neutrophil accumulation has been difficult to examine because the most available antibodies to adhesion molecules, produced against human endothelium, do not cross-react with endothelia from most experimental species. Munro et al., however, recently used immunohistochemical techniques to examine the expression of ELAM-1 and ICAM-1 in the skin of baboons, an experimental species in which substantial cross-reactivity with human antibodies occurs (29). IL-1 and TNF caused induction of ELAM-1 on the surface of venular endothelium in vivo 2 h after intradermal injection, and this induction correlated with the earliest evidence of leukocyte adhesion. Similarly, ICAM-1 expression began to increase 6 to 9 h after intradermal injection of TNF, correlating with the beginning influx of lymphocyte and monocytes. At 24 to 48 h after the injection of IL-1, there was a substantial accumulation of lymphocytes and monocytes, correlated with strong reactivity for endothelial ICAM-1. These results resemble the in vitro findings (29). However, these are two important differences. First, unlike the situation in vitro, there was persistent expression of ELAM-1 after 24 h of TNF injection in these baboons, together with a continuing influx of neutrophils. Although a number of factors may explain this discrepancy, the point reemphasizes the need for caution in extrapolating from the in vitro data to what occurs in vivo. Second, lymphocyte adhesion occurs substantially later in vivo than has been observed in vitro. Further analysis of the role of these adhesion molecules must await studies in which endothelial adhesion can be blocked by specific antibodies or competitive inhibitors in experimental models of inflammation.

In contrast to these proinflammatory actions of IL-1 and TNF in stimulating adhesion, there is evidence that certain cytokines, under the same circumstances, may have antiadhesive effects. Wheeler et al. have shown that, upon activation with IL-1, TNF, or endotoxin, human umbilical vein endothelial cells secrete a soluble inhibitor of leukocyte adhesion (leukocyte adhesion inhibitor) that decreases the hyperadhesive interaction characteristic of IL-1-stimulated endothelium (30). Leukocyte adhesion inhibitor is a protein synthesized by endothelial cells in response to these stimuli and, when isolated and chemically sequenced, was found to be a form of interleukin-8 (IL-8) (31). This cytokine, originally isolated from activated macrophages as a neutrophil chemotactic factor or a neutrophil activating factor (NCF or NAF), is induced to be synthesized and secreted by endothelial cells in response to IL-1, TNF, and endotoxin (32, 33). IL-8 is also a lymphocyte chemoattractant and induces both neutrophil and lymphocyte infiltration of tissues when injected locally (34). How the endothelial antiadhesive effects of this molecule can be reconciled with its neutrophil chemotactic and inflammatory properties is currently under study. In addition to IL-8, transforming growth factor β inhibits cytokine-induced endothelial adhesion in vitro (35).

Before we leave the subject of cytokine effects on endothelial leukocyte adhesivity, two points must be emphasized. The first is that certain mediators (e.g., complement peptides) may affect adhesion by acting on leukocytes, principally by activating the leukocyte adhesion molecules comprising the CD11/18 com-
plex (36–38). TNF itself also activates the CD11/18 complex, eliciting an immediate leukocyte-dependent increase in adhesion, in addition to the ELAM-1-dependent adhesion at 4 to 6 h (39,40). The second point to make is that certain noncytokine mediators, such as histamine and thrombin, while not causing induction of adhesion molecule synthesis, may act rapidly on endothelial cells by causing redistribution and surface expression of adhesion molecules, as noted above in the case of GMP-140. The endothelial cytokine effects must thus be viewed as parts of a sequence of time-dependent events which can modulate leukocyte adhesion in inflammation (41).

**CYTOKINE EFFECTS ON COAGULATION**

The first effect of IL-1 on endothelium to be discovered was concerned with the ability of this cytokine to modulate endothelial cell coagulant properties (42). The normal endothelial cell surface is unable to activate the intrinsic or extrinsic clotting pathways and provides two anticoagulant mechanisms; these cells express thrombomodulin and secrete protein S, thus catalyzing the activation of protein C, and they possess surface-bound anticoagulant heparan sulfate, thus catalyzing the anti-thrombin III pathway (43). Endothelial cells can also initiate the lysis of fibrin clots by catalyzing the activation of plasminogen to plasmin (44). It was first shown that IL-1 markedly increases tissue factor-like procoagulant activity in cultured human umbilical and saphenous vein endothelial cells, which then acquire the capacity to bind factor VIIIa and to initiate the extrinsic clotting pathway (2,42,45). TNF has the same effect (45–47). The effect is transient, with peak expression of tissue factor at 4 to 6 h, and requires RNA and protein synthesis. IL-1 and TNF also caused a decrease in endothelial surface thrombomodulin (45,47), thus markedly inhibiting the anticoagulant effects of protein S and protein C. The effect of thrombomodulin expression is sustained in the continued presence of the cytokines. Both of these effects tend to tip the balance of the coagulant/anticoagulant molecules toward fibrin deposition and intravascular coagulation. Additionally, IL-1 and TNF markedly increase the synthesis of plasminogen activation inhibitor 1 (48–50), potentially diminishing endothelial cell-mediated fibrinolysis. These effects on the coagulation system are dependent on the basal state of endothelium. For example, motile endothelial cells, such as those which undergo regeneration, are more susceptible to cytokine stimulation than are resting endothelial cells (51). In addition, other tumor cell-derived cytokines can directly induce tissue factor expression and act synergistically with TNF.

The only potentially antithrombotic effect of cytokines on endothelium is the influence of IL-1 and TNF on platelet function by augmenting the capacity to synthesize PGI2 (52), a potent inhibitor of platelet aggregation (but not adhesion). This is most obvious when IL-1-induced cells are exposed to an additional stimulus, for example, thrombin or histamine (53). Since IL-1, by increasing tissue factor expression, leads to thrombin generation in situ, marked enhancement of stimulated PGI2 synthesis would be expected to result from IL-1 or TNF treatment in vivo. How the balance between the antiplatelet effect on PGI2 stimulation and the procoagulant effects of IL-1 or TNF on endothelium is resolved in vivo is at present unclear.

Although the actions of IL-1 and TNF on endothelium are very similar, the cytokines do not compete for the same cell surface receptor and their effects are additive in most systems. (Endotoxin also acts directly on endothelial cells and shows activities similar to those of IL-1 and TNF [reviewed in ref. 11]. Endotoxin causes endothelial cells to secrete IL-1 and other cytokines.) There are, however, some differences between the effects of TNF and IL-1. TNF, for example, but not IL-1 causes an increase in the expression of class I major histocompatibility complex (MHC) antigens (54) and can activate neutrophils (39,55).

**CYTOKINE-ENDOTHELIAL INTERACTIONS IN IMMUNITY**

The third important effect of cytokines on endothelium is in immunity and has been deduced from studies of the endothelial effects of IFN-γ. This cytokine is a 40-kDa homodimeric glycoprotein that is secreted by activated T cells and that has a number of actions on effector cells of the immune response. It is a potent macrophage-activating factor, a necessary maturation factor for cytolytic T lymphocytes, and causes B-cell maturation and differentiation.

IFN-γ has numerous effects on endothelial cells in culture. IFN-γ uniquely causes endothelial cells to express class II MHC antigens (56). The effect begins after 6 to 8 h but continues to increase and is maximal after 4 to 6 days. IFN-γ-treated endothelial cells are capable of accessory cell function, and the expression of class II MHC antigens is necessary to permit allogeneic endothelial cells to activate T lymphocytes [reviewed in ref. 57]. To date, IFN-γ appears to be the only cytokine that increases the expression of class II antigens. It also causes increased expression of class I MHC antigens, which are present on endothelium under normal conditions. Class I antigen stimulation, however, is also induced by IFN-α, IFN-β, TNF, and lymphotoxin (58,59). Class I MHC antigens are important in the recognition of foreign antigens by cytolytic T lymphocytes. IFN-γ also causes increased endothelial expression of ICAM-1.
Cytokine induction of endothelial-derived cytokines

Of increasing interest is that endothelial cells themselves are capable of cytokine synthesis and secretion when stimulated by endotoxin and other cytokines. For example, IL-1 causes endothelial cells to synthesize IL-1 (66), monocyte-macrophage colony-stimulating factor (67), granulocyte colony stimulating factor (67,68), IL-6 (69), and IL-8 (32,33). All of these endothelial-derived cytokines can activate various functions of leukocyte populations, but their precise role in vivo in inflammation has not been unravelled. Recently, it has been shown that IL-1 also induces synthesis of a cytokine, called monocyte chemotactic protein (MCP-1), which acts as chemotactic agent for blood monocytes (70-72).

Tables 2 and 3 list the major effects induced by IL-1, TNF, and IFN-γ in vitro. We shall now turn to the relevance of these cytokine-induced alterations in vivo.

The role of endothelial activation in vascular injury in vivo

A great deal of evidence now suggests that the cytokine-endothelial interactions in vitro described above may play a role in a number of pathological processes, as well as in disorders associated with vascular injury. The four most fruitful approaches in studying in vivo endothelial activation have involved the following: examination of the affects of local or systemic injections of recombinant cytokines in experimental animals or in humans (the latter in studies where cytokines are used as therapeutic agents); immunohistochemical detection of endothelial activation antigens (such as ELAM-1, ICAM-1, and ICAM-110/VCAM-1) in animal or human tissues; measurement of intravascular coagulation factors that are influenced by the action of cytokines on endothelium; and the search for antiendothelial antibodies. Here we shall review selected examples of pathological processes to which endothelial-cytokine effects may contribute.

Septic shock

Numerous studies have shown that TNF is a central mediator of shock induced by gram-negative sepsis (reviewed in ref. 73). Infusions of TNF into experimental animal models, including baboons, mimic many of the hemodynamic and vascular changes of septic shock, including hypotension, coagulopathy, leukocyte aggregation, and vascular leakage. Although the cascade of mediators induced in septic shock and their effects on a variety of cell types are complex, it is likely that one major effect of TNF in sepsis is related to its effect on endothelial cell func-

TABLE 2. Principal endothelial actions of IL-1/TNF

<table>
<thead>
<tr>
<th>Action</th>
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<tbody>
<tr>
<td><strong>Increased Leukocyte Adhesion (ELAM-1, ICAM-1, ICAM-110/VCAM-1)</strong></td>
</tr>
<tr>
<td><strong>Enhanced Thrombogenicity</strong></td>
</tr>
<tr>
<td><strong>Increased tissue factor</strong></td>
</tr>
<tr>
<td><strong>Decreased thrombomodulin</strong></td>
</tr>
<tr>
<td><strong>Increased IPA inhibitor</strong></td>
</tr>
<tr>
<td><strong>Increased PGI2 Synthesis</strong></td>
</tr>
<tr>
<td><strong>Increased PDGF Synthesis</strong></td>
</tr>
<tr>
<td><strong>Morphological Changes</strong></td>
</tr>
<tr>
<td><strong>Angiogenesis</strong></td>
</tr>
<tr>
<td><strong>Stimulation of Cytokine Secretion (IL-1, IL-6, GM-CSF, G-CSF, NCF (IL-8), MCP-1)</strong></td>
</tr>
</tbody>
</table>

TABLE 3. Principal endothelial actions of IFN-γ

<table>
<thead>
<tr>
<th>Action</th>
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<tbody>
<tr>
<td><strong>Induction of Class II MHC Molecules</strong></td>
</tr>
<tr>
<td><strong>Increased Expression of Class I MHC Molecules</strong></td>
</tr>
<tr>
<td><strong>Increased Expression of ICAM-1</strong></td>
</tr>
<tr>
<td><strong>Induction of &quot;HEV&quot; Antigens (e.g., MECA-325)</strong></td>
</tr>
<tr>
<td><strong>Increased Lymphocyte Adhesion</strong></td>
</tr>
<tr>
<td><strong>Morphological Changes</strong></td>
</tr>
<tr>
<td><strong>Inhibition of Cell Growth</strong></td>
</tr>
<tr>
<td><strong>Enhancement of TNF-Induced Activation</strong></td>
</tr>
</tbody>
</table>
tions, such as increased surface thrombogenicity and increased leukocyte adhesion.

Evidence of endothelial cell activation in septic shock comes from studies done in collaboration with Tracey, Cerami, and their associates, in which we showed—by immunocytochemical techniques—induction of endothelial ELAM-1 expression in baboons perfused with live Escherichia coli to induce septic shock (74,75). In these experiments, there was extensive endothelial-specific expression of this adhesion molecule in the endothelium of the lung, liver, skin, adrenal gland, connective tissue, and glomerular and peritubular capillaries. In the lung in particular, staining was associated with adherent neutrophils (Cotran et al., unpublished observations). In this model, the development of septic shock can be inhibited by pretreating the animals with antibodies to TNF (74), and, in a limited study, this inhibition was accomplished by abrogation of ELAM-1 staining in endothelium. In current studies done in collaboration with Redl and his associates (Redl et al., unpublished observations), ELAM-1 staining in baboons with septic shock was confirmed and it was further shown that endothelial activation was less pronounced in traumatic hypovolemic shock.

The endothelial abnormalities induced in septic shock or by infusions of TNF can be due either to direct effects of TNF on the endothelium or to indirect effects of TNF on leukocytes. TNF induces leukocyte aggregation, adhesion, and activation and, through the release of oxygen-free radicals or proteolytic enzymes, may cause or enhance endothelial cell lysis or detachment (76). There is indeed evidence that cytokine-treated endothelial cells are more susceptible to lysis by neutrophils. For example, Varani et al. showed that pretreatment of rat pulmonary arterial endothelial cells with TNF or IL-1 increases their sensitivity to killing by neutrophils stimulated with activating agents (77). There is, however, both in vivo and in vitro evidence that TNF increases endothelial permeability directly, without neutrophil participation. Horvath et al., for example (78), found that intravenous infusion of recombinant human TNF in sheep made neutropenic with hydroxyurea, had similar increases in vascular permeability to control sheep with normal white cell counts. Brett et al. (79) found that TNF in vitro increases the permeability of endothelial cell monolayers directly by a mechanism involving a pertussis toxin-sensitive G protein.

Shwartzman Phenomenon

The disseminated intravascular coagulation characteristic of severe gram-negative bacterial infection results in ischemic damage to various organs and can be replicated in experimental animal models by the Shwartzman reaction. In this reaction, two sublethal intravenous injections of endotoxin given 24 h apart in rabbits induce generalized fibrin thrombi which are particularly apparent in glomerular capillaries. In the local Shwartzman reaction, the first dose is injected intradermally and the second is given intravenously. After the second dose, there is virtually immediate leukocyte aggregation and thrombosis, developing at the injection site. In both reactions, leukocytes appear essential to the development of the response.

There is evidence that TNF and IL-1 may in part mediate the Shwartzman reaction. In mice, a Shwartzman-like localized tissue necrosis can be induced by an intradermal injection of endotoxin followed 24 h later by TNF (80). In this model, neither TNF nor IL-1 can replace endotoxin as the priming agent. In rabbits, however, the combination of IL-1 and TNF administered as a single injection can replace endotoxin in priming the local Shwartzman reaction (81). The precise mechanism by which IL-1 and TNF mediate endotoxin-induced Shwartzman reactions is still unclear, but, as previously noted, all three mediators promote endothelial thrombogenicity in vitro by stimulating tissue factor surface expression, inhibiting thrombomodulin expression, and dampening of fibrinolysis. All three also increase leukocyte adhesion. In culture, the effects of these mediators are additive and it is possible that endotoxin primes by increasing endothelial sensitivity to the procoagulant and proadhesion actions of TNF, either directly or through the induction of another cytokine. The complement system, specifically C5a, and neutrophil activation, also appear to be involved in the second phase of the Shwartzman phenomenon (82).

Recent evidence also suggests that Shwartzman-like reactions may play a role in clinical settings in which tissue injury does not involve endotoxin—such as systemic lupus erythematosus (SLE), and cytokine-endothelial effects may be critical in these lesions also (82).

Endothelial Activation in Immune Reactions

The first clear suggestion that cytokine-induced endothelial activation occurs in vivo in humans was the demonstration by immunoperoxidase techniques of induced ELAM-1 expression in an elicited human delayed hypersensitivity skin reaction (83). Both ICAM-1 and ICAM-10/VCAM-1 have since also been shown to be increased in the endothelium of venules in such reactions (25,29). Indeed, cytokine actions of endothelial cells may contribute to several aspects of delayed hypersensitivity. IFN-γ, for example, induces endothelial class II MHC molecule
expression, and it has been shown that the earliest events in delayed hypersensitivity reactions (in guinea pigs) include endothelial class II expression (84) and enrichment of the lymphocytic infiltrates at early times for antigen-specific T cells (85). These observations suggest that endothelial cells could be presenting foreign antigen to T cells. Endothelial cells in vitro can actively increase the quantity of IL-2 produced by activated T cells (86), an event which is dependent on cell-cell contact involving CD2/LFA-3 interactions (87). This endothelial cell enhancement of T cell IL-2 secretion may be critical to the ability of endothelial cells to elicit primary T cell antigen responses that may have consequences in the development of cell-mediated immune reactions.

In addition to induction of endothelial class II expression in delayed hypersensitivity reactions, cytokines may also be involved in the early phase of neutrophil infiltration through induction of ELAM-1 and in the mononuclear cell adhesion and infiltration through induction of ICAM-1/VCAM-1 or ICAM-1. Finally, cytokines may also be responsible for the morphological changes and the venular leakage to macromolecules which occur at the peak of cell-mediated skin reactions, since morphological changes in and increased permeability of endothelium can be regularly produced in vitro by IL-1 and TNF in vitro (see above). Thus, cytokine-inducible changes may account, at least in part, for several steps of delayed hypersensitivity reactions, including T cell recognition of antigen activation, increased permeability to macromolecules, adhesion and emigration of leukocytes, and the characteristic morphological changes of hypertrophy of endothelial cells.

Endothelial activation antigens have also been identified in a variety of other human immunologically mediated lesions (88), such as erythema multiforme, photoallergic eruptions, bullous pemphigoid, cutaneous vasculitis, allograft rejection (89), and the late-phase allergic reaction typical of atopic dermatitis (90). In each of these conditions, the lesions are associated with an active inflammatory infiltrate and are almost certainly immunologically mediated. However, the source of the cytokines and the precise role of the cytokine-endothelial changes in each of these conditions has not yet been fully investigated and deserves further study.

Endothelial Activation in Immune Vasculitis

One final example of cytokine-endothelial alterations which may contribute to vascular injury is that which may occur in certain autoimmune vasculitides. The possibility that immune injury to endothelium may occur in vasculitis has been the subject of much speculation, and, indeed, circulating antiendothelial antibodies have been reported in several connective tissue disorders (91). For example, sera from patients with active SLE bind to cultured human umbilical vein endothelial cells, via the Fab immunoglobulin (IgG) domain (92). Circulating IgG antibodies that bound with high affinity to human umbilical vein endothelium can be found in 70% of patients with SLE, 30% of those with scleroderma, and 28% of those with rheumatoid arthritis (93). In such studies, there was no evidence for cytotoxicity induced by antiendothelial IgG in the presence of complement. More recently, circulating cytotoxic antibodies that reacted with monocyes and endothelial cells were reported in a variety of vasculitides (94).

Studies on the vasculitis of Kawasaki disease suggest a novel mechanism of antibody-mediated injury to endothelial cells, involving cytokine-induced endothelial activation. Kawasaki disease is an acute illness of unknown cause, affecting infants and young children, and is associated with vasculitis, primarily affecting the coronary arteries in about 20% of patients. Myocardial infarction and sudden death or chronic coronary insufficiency may result from coronary artery aneurysm. Immunoregulatory alterations are present in patients with acute Kawasaki disease, including increased numbers of activated helper T cells and monocytes, deficiency of suppressor/cytotoxic T cells, and polyclonal B cell activation (95). The condition is also associated with elevated circulating levels of IL-1 (96), TNF (97), and IFN-γ (98). Immune complexes are present in the circulation but occur late and do not correlate with disease activity.

Children in the acute phase of Kawasaki disease syndrome possess in their sera at least two types of antibodies which lyse activated endothelium (99,100). The first lyses endothelial cells treated with interferon for 1 to 3 days but not control endothelium, control or interferon-treated fibroblasts, or control or IFN-γ-treated smooth muscle cells. The second set of antibodies lyses IL-1- or TNF-treated endothelial cells, but not control endothelial cells, control or cytokine-treated fibroblasts or smooth muscle cells. The time course of induction is maximal at 4 to 6 h after IL-1 or TNF treatment, disappearing by 24 h. The two antibodies recognize altogether different antigens as shown by cross-adsorption studies. These studies led to the hypothesis that T cell and monocyte activation in Kawasaki disease results in cytokine secretion, endothelial activation, and the induction of novel endothelial antigens. These activation antigens then evoke an antibody response to these new surface antigens, resulting in cell lysis. This hypothesis is supported by recent studies which examined patients with Kawasaki disease subjected to intravenous gamma globulin therapy (101). This treatment prevents the development of coronary aneurysms and ameliorates symptoms and signs of the
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disease, including the fever and the skin rash. In the first study, peripheral blood mononuclear cells from gamma globulin-treated patients were shown to produce significantly less IL-1 than do such cells before therapy. In the second, skin biopsies were examined by immunoperoxidase techniques for the presence of the activation antigens ELAM-1 and ICAM-1. Such antigens were present or accentuated in all biopsies prior to gamma globulin therapy but either completely disappeared or were markedly diminished in biopsies of patients responding to therapy. The association between the reduction of cytokine secretion and reversal of endothelial cell activation on the one hand and evidence of vasculitis on the other support a role for immune-mediated injury to cytokine-induced endothelial activation antigens in endothelial cell lysis. The nature of the endothelial activation antigens in Kawasaki disease and the sequence of events leading to endothelial cell lysis to necrotizing vasculitis are unknown. It is possible, however, that in Kawasaki disease and in similar conditions, cytokine-induced activation and resultant immune endothelial injury may initiate the mural vasculitis which ensues.

By using the same techniques, Leung et al. have found complement fixing antibodies which lyse cultured human umbilical vein endothelial cells in sera from patients with acute hemolytic uremic syndrome (102). These antibodies, however, lyse only normal cultured human endothelial cells but not cells that have been treated with the cytokine IFN-γ. The study raises the possibility that the cytokine induces the loss of a specific class of an antigen or an alteration in the structure or accessibility of endothelial cell surface molecules. Curiously, only three of the five adult patients with acute nonrelapsing thrombotic thrombocytopenic purpura had lytic and endothelial antibodies and only one of these recognized an antigen lost upon exposure to IFN-γ.

SUMMARY AND CONCLUSIONS

We have summarized the evidence that cytokines can activate endothelial cells in numerous ways and that such activation is an important component of immune and inflammatory reactions. IL-1 and TNF in particular can increase endothelial adhesiveness to leukocytes, which has led to the discovery of endothelial adhesion molecules. These adhesion molecules belong to distinct structural families of proteins—including the selectins (ELAM-1 and GMP-140)—characterized by a lectinlike domain on the amino acid terminus and proteins belonging to the immunoglobulin gene superfamily (ICAM-1, ICAM-2, and ICAM-110/VCAM-1). Their surface expression is time dependent. IL-1 and TNF influence adhesion of all leukocyte cell types: IFN-γ appears to be selective for lymphocytes, although it synergizes with TNF in inducing ELAM and ICAM-1. IL-1 and TNF have profound effects on the endothelial coagulant-anticoagulant balance and enhance surface thrombogenicity and intravascular coagulation. These cytokines have a number of other effects on endothelium which are largely proinflammatory. Cytokines stimulate endothelial cells to synthesize and secrete a variety of other cytokines including IL-1, IL-6, IL-8, and MCP-1. These endothelial-derived cytokines almost certainly have roles to play in the evolution of inflammatory and immune reactions, but these remain to be determined. The effects of IFN-γ on endothelium have important roles in immune responses, reflected by the ability of IFN-γ to induce human lymphocyte class I and class II antigens, lymphocyte adhesion and transmigration, enhancement of IL-2-driven T cell proliferation, and acquisition of morphological properties of high endothelial venules.

Although most of the cytokine effects on endothelium have been studied in culture, there is sufficient evidence from both animal and human studies that they occur in vivo, after local and systemic cytokine injections, and in a number of pathological conditions. In particular, cytokine-endothelial interactions are implicated in septic shock, the Shwartzman phenomenon, the vascular and cellular events of delayed hypersensitivity reactions (including transplantation), and certain forms of autoimmune vasculitis. In addition, the study of such cytokine-endothelial effects has provided new insights as to how inflammatory and immune reactions develop and points to new areas where therapeutic intervention may influence these responses.

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REFERENCES

6. Cavender DE, Haskard DO, Joseph B, Ziff M: Interleukin 1 increases the binding of B and T lym-
Pohlman TH, Stanness KA, Beatty PG. Ochs


37. Arnaout AM, Lanier LL, Faller DV: Relative con-
Cytokine-Endothelial Interactions

- Bevilacqua MP, Pober JS, Majeau GR, Cotran RS. The role of endothelial cells in inflammation. Transplantation 1990, in press.

- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


- Pober JS, Cotran RS: The role of endothelial cells in inflammation. Transplantation 1990, in press.


