EXPRESSION OF MAJOR HISTOCOMPATIBILITY CLASS II ANTIGENS BY VASCULAR ENDOTHELIAL CELLS LEADS TO AMPLIFIED IMMUNOINFLAMMATORY PROCESSES

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Vascular endothelial cells (ECs) have been demonstrated to play crucial roles in inflammation and immune responses by expressing various adhesion molecules, such as MHC class II antigens, ICAM-1 and ELAM-1 (E-selectin), and by synthesizing various cytokines including IL-1, IFN- γ and TNF. They act as antigen presenting cells in leading to a cascade of immunologic and inflammatory events. The expression of cell adhesion molecules by ECs, particularly high endothelial venules, mediates permeation of lymphocytes and leucocytes in inflammatory lesions, and subsequently leads to continuous inflammation. Both morphological and functional alterations of ECs named "activation" are induced by cytokines *in vitro* and inflammatory or immune responses, and the concept of endothelial activation is useful in understanding endothelial cell functions. In addition, the immunoperoxidase technique is useful for studying morphological and functional changes of ECs in inflammatory and immune diseases.

It is generally recognized that vascular endothelial cells (ECs) constitute the first line of contact between the host and antigenic substances in the blood circulation, and make their own unique contribution to the immune network (1, 5, 24). Recognition of an antigen by primed T cells requires the participation of some type of major histocompatibility complex (MHC) class II (Ia) antigen-positive antigen-presenting cells (APCs). It is clear that MHC class II antigens are expressed on the EC surface, and the antigen presentation by ECs is a change leading to a cascade of immunologic events (8, 25). In addition, coupled with the MHC class II antigen expression, ECs, under certain circumstances, are capable of producing cytokines and cell adhesion molecules which have been shown to have marked effects on adhesiveness of lymphocytes or leucocytes and their cell-surface antigen expression (6, 21, 30).

The intent of this review is to introduce and summarize several current informations of how ECs play a role in a cascade of immunologic or inflammatory processes, and to emphasize that immunocytochemical techniques have created several important ideas in the

endothelial research.

Heterogeneous expression of MHC class II antigens on endothelial cells

It has been well documented that ECs are involved in hemostasis by synthesizing various kinds of coagulant factors including Willebrand factor antigen (10, 20). Moreover, ECs share many kinds of immunological properties as APCs expressing MHC class II antigens and producing interleukin 1 (IL-1) and other cytokines (8, 25, 30) (Fig. 1a, b), and also possess an additional role in local immune responses by regulating emigration of lymphocytes from the blood circulation into lymphoid tissues or sites of inflammation expressing a series of cell adhesion molecules (3, 6, 21, 22). Thus, for the immune system to function properly, ECs may play crucial roles. We have investigated the phenotype and immunological roles of ECs by the immunocytochemical technique, and clearly demonstrated that ECs in each anatomical compartment in the liver, lung, kidney and intestine possess heterogeneous phenotypic or immunologic properties (12, 19, 31). This may reflect functional differences of ECs in different anatomical compartments as well as disease processes.

In the liver, for example, MHC class II antigens, such as HLA-DR antigen, and IL-1 are expressed by

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FIGS. 1a, b. Immunohistochemical localization of HLA-DR(a) and IL-1(b), on the vascular endothelial cells. These antigens are localized on both luminal and basal surfaces and in pinocytotic vesicles along the plasma membrane.

almost all ECs including the sinusoidal EC, whereas a monoclonal antibody (MoAb), OKM5, which detects antigenic determinants distributed on the functionally distinct peripheral blood APC subsets (CD36) (13), reacts only with the sinusoidal ECs, but does not with ECs in the portal area and of the central vein (19). MoAb OKM5 is not reactive with the liver sinusoidal ECs associated with pericellular fibrosis along hepatocytes (7). By contrast, the von Willebrand factor is not synthesized by sinusoidal ECs in normal liver but always found in ECs in the portal area and central vein (19). In cases with the pericellular

fibrosis the sinusoidal ECs turn positive for von Willebrand factor (7). In the lung, capillary ECs in the alveolar septa also possess both HLA-DR antigen and IL-1, but do not von Willebrand factor (32). In thickened alveolar septa in pulmonary fibrosis, however, HLA-DR antigen and IL-1 are hardly detected, whereas von Willebrand factor is synthesized (15). It is reasonable to speculate that ECs in the alveolar septa are first injured by some antigenic stimulation, and that ECs with different phenotypic properties are regenerated coupled with fibrogenesis. These regenerated ECs may be involved in coagula-

tion instead of immunologic functions. Of particular interest for MHC class II antigens involved in organ transplantation are their expressions in ECs (11, 25). We demonstrated immunocytochemically that HLA-DR antigen is expressed in ECs of the glomerular tuft and peritubular capillaries (12), which are first injured at the rejection of transplanted kidney, but not in most large vessels of the kidney. It has been demonstrated that a soluble factor(s), such as interferon (IFN), derived from activated T lymphocytes can induce the expression of MHC class II antigens in HLA-DR-negative endothelial cells as well as epithelial cells in the intestinal tract, and also can enhance the expression of HLA-DR antigen in HLA-DR-positive cells of a monocyte-macrophage lineage (4, 26). Although the biological implication of the induction of MHC class II antigens on endothelial cells and epithelial cells are still unclear, it has been shown that these cells induced to express MHC class II antigens can serve as targets for MHC class II antigen-specific cytotoxic T lymphocytes as shown in human colonic mucosa with ulcerative colitis as well as cultured endothelial cells treated with IFN γ (4, 17, 28). As mentioned above, we and others have studied the expression of MHC class II antigens with MoAbs specific for HLA-DP and DQ antigens, in addition to the HLA-DR antigen (4, 12, 15, 17, 19, 31). MoAbs have been extremely useful for studying the expression of class II MHC antigens on human ECs and mononuclear cells. The immunoperoxidase technique using MoAbs specific for those class II MHC antigens allowed the systemic evaluation of ECs of variety of organs.

Expression cell adhesion molecules on endothelial cells in health and disease

Adhesion of lymphocytes or leucocytes to ECs and their extravasation into perivascular tissues begin with the binding of specific cell surface receptors of these cells to their appropriate counter-receptors of ECs (3, 22). Adhesion of lymphocytes to several APCs is an obligatory step in antigen presentation. A number of molecules, which are involved in both antigen-specific and non-specific contribution to those cell adhesion processes, have been identified. ECs express these adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) (CD54) and endothelial leucocyte adhesion molecule-1 (ELAM-1) (E-selectin) (6, 27) (Figs. 2a, b). Recently class II MHC antigens have been known as an adhesion molecule. These events occur during the normal process of lymphocyte or leucocyte migration as well as during the infiltration of these cells into sites of inflammation (3, 21, 22, 29).

Recent immunohistochemical studies showed that ICAM-1 is a adhesion molecule belonging to immunoglobulin superfamily resembling HLA-DR antigen (29), and is expressed on non-hematopoietic cells such as ECs, certain epithelial cells and fibroblasts, and on hematopoietic cells such as tissue macrophages and germinal center dendritic cells in lymphnodes and Peyer's patches (6, 18). The overall distribution of ICAM-1 in these cells is similar to that of HLA-DR antigen as described (Fig. 3a). It has been well documented that ICAM-1 expression on these cells is increased by either IL-1 and IFN γ , and may play a role in class II MHC antigen-restricted T cell activation (6).

ICAM-1 staining on vascular ECs is most intense in T cell areas in lymphnodes, Peyer's patches and tonsils, particularly in those showing reactive hyperplasia. An inflammatory disorder of intestinal tracts, such as ulcerative colitis, is characterized by the severely inflamed and heavily vasculated mucosa with occasional ulceration (17). Almost all cells in the inflamed mucosa with ulcerative colitis including endothelial cells, fibroblasts, dendritic cells and epithelial cells extensively express ICAM-1 together with HLA-DR antigen (2, 17, 28) (Fig. 3b). These aberrant expressions of ICAM-1 and HLA-DR antigen may be involved in exaggeration of immune reactions in the intestinal mucosa, and subsequently lead to the chronic and continuous inflammation observed in the mucosa with ulcerative colitis.

ELAM-1 belongs to the LECAM family and is rapidly induced in endothelial cells by several inflammatory cytokines including IL-1, IFN γ and tumor necrosis factor (TNF) (16). Recently substance P, which is a major neuropeptide in the intestinal mucosa, has been known to induce ELAM-1 expression (16). It has been well known that ELAM-1 plays an important role in permeation of neutrophils in inflammatory lesions (5, 21, 27, 33). In normal human intestinal mucosa, all capillaries lack ELAM-1 expression, whereas high endothelial venules (HEV) in T cell area of Peyer's patches always express ELAM-1 (21). These ELAM-1-positive HEV may involve the migration of lymphocytes because memory T cells also bind to ELAM-1 (31). Active inflammatory lesions in ulcerative colitis are characterized by abundant ELAM-1-positive dilated venules and heavy infiltrations of granulocytes (21), which bear a sialyl Lewis antigen, the ligand of ELAM-1 (Fig. 2c). Thus, venules in ulcerative colitis actively take part in permeation of inflammatory cells by induction of adhesion molecules to maintain acute inflammation. Furthermore expression of ELAM-1 on venules is reported in rheumatoid

FIG. 2. Immunohistochemical localization of ICAM-1(a) and ELAM-1 (E-selectin) on the vascular endothelial cells. These antigens are localized on both luminal and basal surfaces and in pinocytotic vesicles along the plasma membrane. ELAM-1 is also present in the rough endoplasmic reticulum (ER) and perinuclear space (PS).

TABLE 1. Phenotypic heterogeneity of vascular endothelial cells

	CD36	vWF	ICAM-1	ELAM-1	IL-1	HLA-DR
capillaries	+	—	— ~ +*	—	+**	+**
venules	— ~ +*	+	+	+	+**	+**

* active inflammatory areas

** not always expressed

arthritis known as a chronic inflammation in synovial tissue (14). Table 1 summarizes heterogenous expression of antigens related to ECs.

Roles of the endothelium in inflammation and immunity—a concept of endothelial activation

Endothelial cells play crucial roles in the inflammatory and immune responses. Immunohistochemical studies have elucidated that certain ECs carry the surface antigens common to cells of a macrophage-monocyte lineage and there is the phenotypic heterogeneity among vasculatures in various tissues and disease states (1, 5, 8, 12, 15, 19, 24, 32). As mentioned above, inflammatory infiltrations and lymphocyte migrations are carried out by the venules of the inflamed tissue and lymphoid tissue

respectively (3, 14, 21, 22, 28). The endothelial lining cells of these venules become plump (hyper-trophied) and protrude into the lumen (20, 21).

Our ultrastructural examination revealed that they display increased quantities of biosynthetic organelles such as rough endoplasmic reticulum (rER). Immunoelectron microscopically, von Willebrand factor is localized in these well-developed rER, vesicles and Weibel-Palade bodies, suggesting active protein production (20). In certain inflammatory lesions, or by IFN- γ stimulation *in vitro*, class II MHC antigens are induced on ECs, and they can stimulate a proliferative response by allogenic peripheral blood lymphocytes (1, 9, 26). This is a reaction dependent on recognition of foreign class II MHC antigens by helper T cells. Furthermore, ECs produce predomi-

FIGS. 3a-c. Immunohistochemical localization of ICAM-1 in normal colonic mucosa(a) and inflamed colonic mucosa from an ulcerative colitis patient(b), and ELAM-1 (E-selectin) on dilated venule containing leucocytes in the colonic mucosa from an ulcerative colitis patient(c). For details, see text.

FIG. 4. A schematic illustration of the mechanism of "endothelial cell activation" by cytokines. For details, see text. (from ref. 24)

nantly membrane-associated IL-1 bioactivity in response to TNF, and expression of adhesion molecules by ECs is induced by IL-1, $\text{INF } \gamma$ and TNF (6, 24). These effects on ECs induced by several well-characterized cytokines precisely defined *in vitro* systems, and also occur *in vivo* (24). The expression of ELAM-1 and interleukin-8 (IL-8) potentially regulates the avidity of neutrophil binding since IL-8 may alter the ELAM-1 counter-receptor (33). We demonstrated the histological evidence for ELAM-1 expression without leucocytes without leucocyte-adhesion to ECs (21), arguing that some regulatory events take place. These findings are appealing in view of its signaling role in ECs in immuno-inflammatory responses (33).

From several reported studies cited in this review by the authors and others, it has been clarified that immune and inflammatory responses are initiated by T cell recognition of a foreign antigen in the tissue space, and the relevant T cells secrete IL-2 and $\text{INF } \gamma$, which activate local venular ECs to express class II MHC antigens and ICAM-1, and also to secrete IL-1. This bestows increased capacity to present foreign antigens to additional T cells, and thereby T cell activation, causing secretion of more cytokines, and thus amplifying and maintaining immune and inflammatory responses. In this occasion the morphological

changes of ECs are induced by cytokines. Thus the concept of "endothelial activation" includes both morphological changes and modulation of endothelial functions (Fig. 4). Pober (24) emphasized in his lecture that the concept of "endothelial activation" has been extremely useful in understanding endothelial cell functions and active roles of ECs in the local inflammatory and immune diseases.

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