

P-SELECTIN AND VON WILLEBRAND FACTOR IN BOVINE MESENTERIC LYMPHATICS: AN IMMUNOFLUORESCENT STUDY

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ABSTRACT

P-selectin (PADGEM, GMP-140, CD62) is an integral membrane protein specific to alpha granules of platelets and Weibel-Palade bodies of blood vascular endothelial cells. The presence in lymphatic endothelial cells of numerous Weibel-Palade bodies and their positivity to immunocytochemical reaction for von Willebrand factor have previously been characterized and described. Because von Willebrand factor and P-selectin codistribute in Weibel-Palade bodies of blood vascular endothelial cells we investigated the presence of both P-selectin and von Willebrand factor in lymphatic endothelium. Lymphatic vessels expressed positive reaction to immunocytochemical assay thereby demonstrating the presence of P-selectin in the endothelium. Distribution and intensity of the reaction were similar to those observed in bovine blood vascular endothelium.

Lymphatic vessels are lined by a layer of flat endothelial cells which are morphologically similar to blood vascular endothelial cells. Nevertheless little is known about the metabolic and functional features of lymphatic endothelial cells and about their possible similarities with blood vascular endothelial cell functional properties.

Blood vascular endothelial cell cytoplasm exhibits numerous Weibel Palade bodies

which contain the adhesive molecule von Willebrand factor (vWf) (1). These organelles are also the intracellular storage site for the adhesion molecule P-selectin (2,3). Von Willebrand protein and P-selectin are synthesized by endothelial cells and megakaryocytes only and are also stored in platelet alpha granules (4,5). Upon stimulation with physiologic agonists such as histamine or thrombin, or experimental secretagogues such as phorbol ester or calcium ionophore, Weibel-Palade bodies fuse with the plasma membrane. This process induces the rapid and dose-dependent release of high molecular weight forms of von Willebrand factor and also a rapid increase in surface expression of P-selectin (6-8). Von Willebrand factor is secreted into the circulation or incorporated in subendothelial extracellular matrix, and is involved in platelet/vessel wall interactions, supporting thrombocyte adhesion and aggregation at the site of vascular injury. Surface P-selectin expression, on the other hand, helps in recruitment of phagocytic cells (neutrophils and monocytes) and mediates the interaction between leukocytes, platelets and endothelial cells of the blood vessel wall (3,9).

The presence in lymphatic endothelial cells of numerous Weibel-Palade bodies and their positivity to the immunocytochemical reaction for vWf factor have been previously characterized and described (10-12). Because vWf and P-selectin codistribute in Weibel-

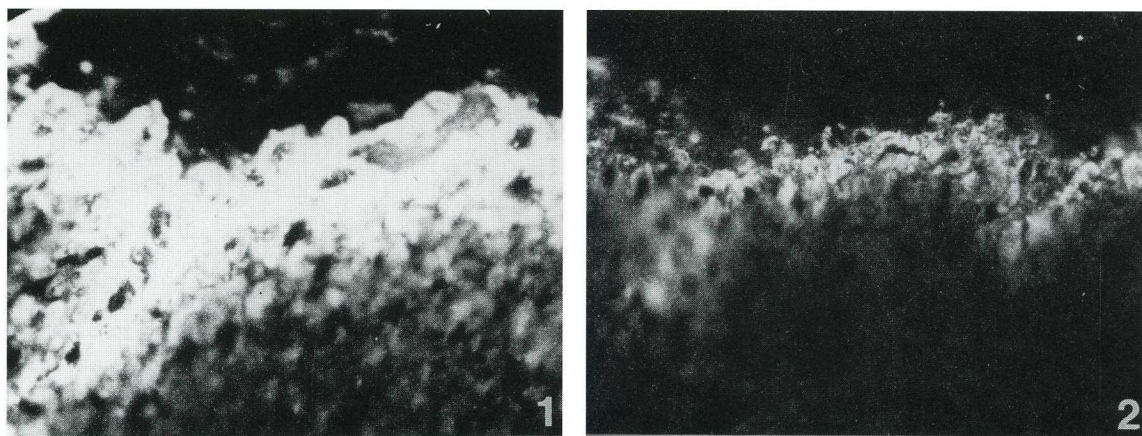


Fig. 1. vWf immunofluorescence reaction. A finely granular positive pattern outlines the endothelial side of bovine venous (1) and lymphatic (2) mesenteric vessels. 50x

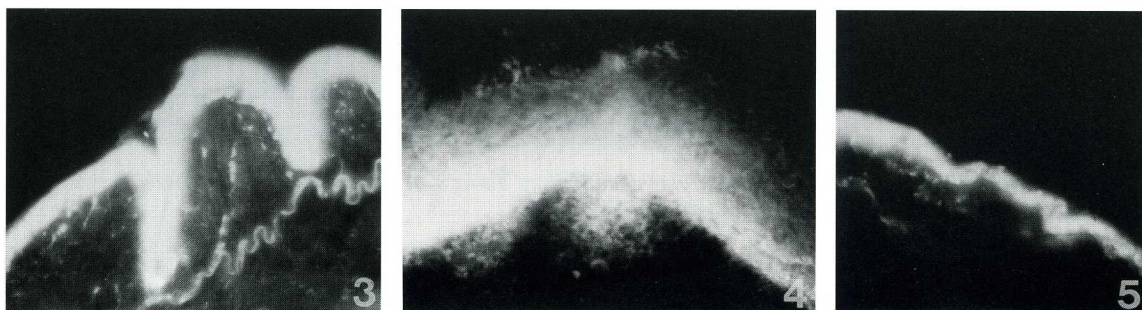


Fig. 2. P-selectin immunofluorescence reaction. An intense continuous positive pattern characterizes the inner side of bovine arterial (3), venous (4) and lymphatic (5) mesenteric vessels. 50x

Palade bodies of blood vascular endothelial cells, we investigated the possible presence also of P-selectin in lymphatic endothelium.

MATERIAL AND METHODS

Lymphatics and blood vessels from bovine mesenteries were obtained at the local slaughterhouse. The lymphatic vessel network was delineated by injecting a solution of 0.1% Evan's blue dye in phosphate buffered saline (PBS) into mesenteric lymph nodes.

Small pieces of lymphatic vessels and blood vessels were dissected and frozen in liquid nitrogen. Cryostatic sections (15 μ m) were fixed for 30 min in a mixture of ethanol

and acetic acid (95% and 5% v/v respectively), and washed in PBS.

Some sections were incubated for 30 min with polyclonal rabbit antibody to vWf (Rabbit anti vWf antibody, Dakopatts) diluted 1:50 in PBS, washed, incubated for 30 min with secondary swine antibody to rabbit IgG (Swine anti-rabbit IgG, Dakopatts) conjugated with tetramethyl rhodamine isothiocyanate (TRITC) diluted 1:100 in PBS. Sections were washed in PBS and mounted in glycerol/PBS (9:1) examined under fluorescence microscopy and photographed, as previously described (13).

Other sections were incubated for 30 min with monoclonal mouse antibody to P-selectin



Fig. 3. Electron micrograph of the flat, irregular endothelium lining a bovine mesenteric lymphatic vessel. Some cross-sectioned Weibel-Palade bodies (arrows) are seen. 30,000x

(Monoclonal antibody to human platelet GMP 140, Takara Biomedicals) diluted 1:320 in PBS, washed in PBS and incubated for 30 min with secondary TRITC-conjugated rabbit antibody to mouse IgG (Rabbit antimouse IgG, Dakopatts) diluted 1:40 in PBS. Sections were washed in PBS and mounted as previously mentioned.

Small pieces of the same lymphatic and blood vessels were dissected and fixed for 60 min in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M Na cacodylate buffer, pH 7.4, at 4°C, postfixed for 60 min in 1.33% OsO₄ in 0.2 M collidine buffer, pH 7.4 at 4°C, dehydrated and embedded in epoxy resin for transmission electron microscopy (TEM).

RESULTS

The immunocytochemical reaction to detect the presence of vWf in the endothelial cells stained positively both in the blood vessels and the lymph vessels (*Fig. 1*).

Moreover, both vessels (lymph and blood) expressed a positive reaction to the immunocytochemical assay demonstrating the presence of P-selectin in the endothelium. We observed a continuous, intense fluorescent staining which labeled the inner layer of the vessel wall. No differences in distribution and intensity of the microgranular pattern of the reaction were observed between venous or arterial and lymphatic vessels (*Fig. 2*).

By ultrastructural examination on thin sections we were able to observe Weibel-Palade bodies among the cytoplasmic organelles of the flat and irregular endothelial cells of the same blood and lymphatic mesenteric vessels (*Fig. 3*).

DISCUSSION

This immunocytochemical technique has demonstrated the presence of vWf and P-selectin in bovine mesenteric lymphatic

endothelium. Ultrastructural study on portions of these lymphatic vessels confirm the presence of Weibel-Palade bodies in the cytoplasm of the endothelial cells. Taken together, these observations suggest that Weibel-Palade bodies are the storage cytoplasmic organelles for vWf and P-selectin in lymphatic endothelium as has already been demonstrated for blood vascular endothelium (1,2). The demonstration of the co-distribution of vWf and P-selectin in blood vascular endothelial cells suggests the presence of important co-regulators for hemostasis and inflammation. Localization of vWf and P-selectin in Weibel-Palade bodies of lymphatic endothelial cells also suggest a functional correlation between these adhesive molecules, although the precise role of P-selectin in lymphatic vessels is still unclear. Perhaps P-selectin acts to regulate white blood cell interaction with lymphatic vascular function.

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