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Exacerbation of Vascular Endothelial Injury in the Generalized Shwartzman Reaction by the Administration of Anti-E-Selectin Antibody

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***Abstract*: Previously, we reported that the consecutive administration of lipopolysaccharide (LPS) into LPS-sensitized mice for the generalized Shwartzman reaction (GSR) induced systemic injury of vascular endothelial cells. The aim of this study was to investigate the participation of vascular adhesion molecules in the vascular endothelial injury of GSR. The administration of anti-E-selectin antibody in GSR-induced mice resulted in massive apoptosis of vascular endothelial cells and congestion in blood vessels. Further, marked hemorrhage was found in the pulmonary alveoli of those mice. GSR, especially lung injury, was definitely exacerbated by the administration of anti-E-selectin antibody. On the other hand, the administration of anti-VCAM-1 antibody did not induce such injury of vascular endothelial cells. The possible role of E-selectin in the exacerbation of vascular endothelial injury in GSR is discussed.**

***Key words*: Endotoxin, Lipopolysaccharide, DIC, Vascular endothelial cell, E-selectin, Anti-E-selectin antibody**

The generalized Shwartzman reaction (GSR) is a potentially lethal shock reaction and induced by two consecutive injections of lipopolysaccharide (LPS) (which are called preparative and provocative injections, respectively) into animals at an interval of 24 hr (3, 4, 14, 18). GSR is characterized by vascular occlusion, hemorrhage, perivascular accumulation of leukocytes, and necrosis (14, 18), and it is known as an experimental disseminated intravascular coagulation model (1, 8). Recently, the injury of vascular endothelial cells is reported to be mediated mainly by apoptosis, but not necrosis (8, 11). The vascular endothelial injury seemed to be caused by the presence of excessive leukocytes (2, 11, 16). The blocking of leukocyte adhesion to vascular endothelium might be a potential therapeutic target to prevent GSR (2, 16). In fact, we reported that anti-ICAM-1 antibody prevented the vascular endothelial injury of GSR (12). The aim of this study was to investigate the role of E-selectin and vascular cell adhesion molecule-1 (VCAM-1) in the prevention of GSR, especially vascular endothelial injury. Incidentally, we found that the administration of anti-E-selectin antibody markedly exacerbated systemic injury of the vascular endothelial cells during GSR. Here, we discuss the possible role of E-selectin on the exacerbation of vascular endothelial injury during GSR.

For the induction of GSR, 6–8-week-old male BALB/c mice (SLC, Hamamatsu, Japan) were injected twice with LPS. LPS extracted from *Klebsiella pneumoniae* O3 LEN-1 by the phenol water method (19, 20) or LPS from *Escherichia coli* O55 (Difco Laboratories, Detroit, Mich., U.S.A.) was used. The optimal dose of LPS (5 μg) was injected intradermally into the footpads of mice as a preparative injection for priming of GSR although typical GSR is produced in susceptible rabbits by two intravenous injection of LPS. A provocative injection of LPS (300 μg) was administered intravenously after 18–24 hr (11). More than 80% of the mice died within 12 hr after the provocative injection of LPS. Three to four mice were used in each experimental group. One-hundred micrograms of rat monoclonal anti-E-selectin IgG_{2a} antibody (PharMingen, San Diego, Calif., U.S.A.) was administered intravenously together

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Abbreviations: GSR, generalized Shwartzman reaction; ICAM-1, intercellular adhesion molecule-1; LPS, lipopolysaccharide; VCAM-1, vascular cell adhesion molecule-1.

with a provocative injection of LPS. Various organs were removed 6 hr after the injection. Histologically, severe injury of vascular endothelial cells and congestion in blood vessels were detected in various organs of mice receiving anti-E-selectin antibody with LPS. Especially, the lung lesions were characterized by marked hemorrhage in the pulmonary alveoli (Fig. 1). Those severe lesions were not found in mice injected with 100 μg of rat monoclonal anti-VCAM-1 IgG_{2a} antibody (PharMingen) in the place of anti-E-selectin antibody. Furthermore, such lesions were seen in neither mice injected with anti-E-selectin antibody alone nor LPS-sensitized mice receiving anti-E-selectin antibody alone. Therefore, the provocative injection of LPS together with anti-E-selectin antibody was required for the exacerbation of GSR. In order to determine whether the injury of vascular endothelial cells was mediated by apoptotic cell death, they were stained with *in situ* nick end-labeling specific for fragmented DNA as described previously (7, 21). A number of nuclei of vascular endothelial cells were stained positively in GSR-induced mice receiving anti-E-selectin antibody, suggesting apoptotic cell death (Fig. 2). The administration of anti-E-selectin antibody caused much more apoptotic cell death of vascular endothelial cells during GSR. Next, the expression of E-selectin was studied in GSR-induced mice with an immunohistochemical staining method using a 1:200 dilution of anti-E-selectin antibody (Fig. 3). A preparative injection of LPS induced a significant expression of E-selectin on vascular endothelial cells although E-selectin was not constitutively present on them. The expression of E-selectin was not altered much by the provocative injection of LPS. In addition, the expression of VCAM-1 was not affected by the administration of LPS.

In this study, we demonstrated that the administration of anti-E-selectin antibody into GSR-induced mice caused the exacerbation of GSR rather than the prevention of it. The lesions were characterized by massive apoptosis of vascular endothelial cells and severe lung injury with hemorrhage. No such lesions were induced by

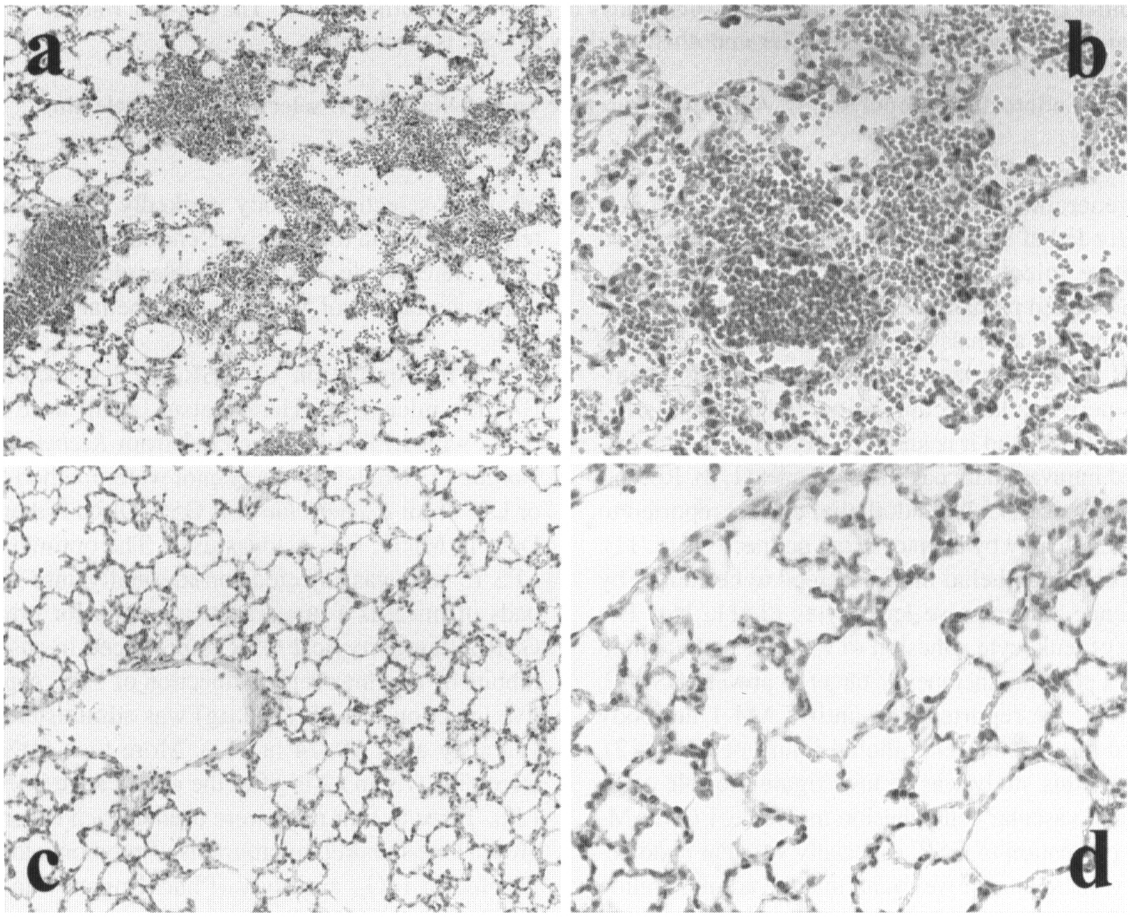

Fig. 1. Histology of the lungs in GSR-induced mice receiving anti-E-selectin antibody. GSR was induced by two consecutive injections of LPS into mice at an interval of 24 hr. Anti-E-selectin antibody (a, b) or anti-VCAM-1 antibody (c, d) was injected together with a provocative injection of LPS. The lungs were removed 6 hr after the injection. Lung sections were stained with hematoxylin and eosin. Marked congestion in blood vessels and hemorrhage into the alveoli (a, b) were seen in mice receiving anti-E-selectin antibody. a and c, ×200; b and d, ×400.

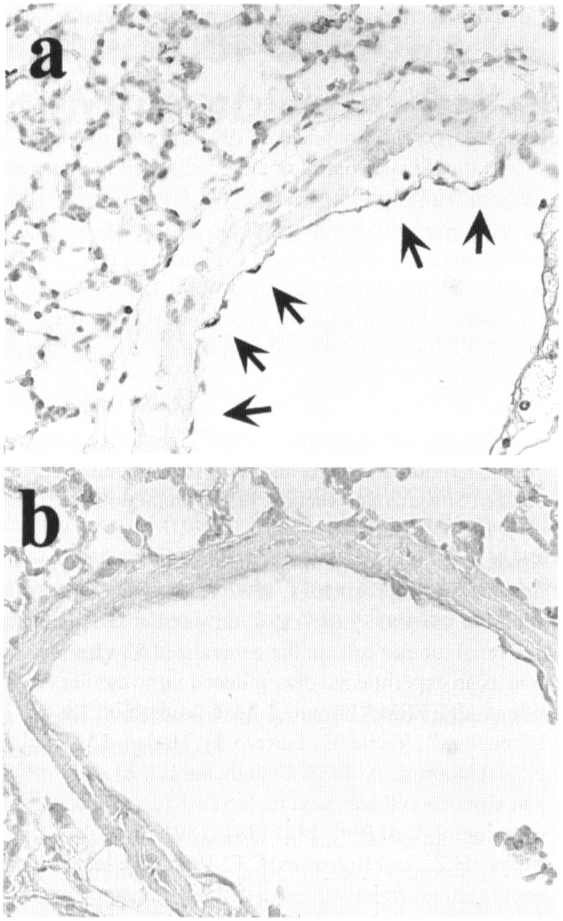

Fig. 2. Apoptotic cell death of vascular endothelial cells in the lungs of GSR-induced mice receiving anti-E-selectin antibody. The lungs were removed 6 hr after the injection and subjected to nick end-labeling specific for fragmented DNA. A number of nuclei of vascular endothelial cells (arrows) are stained positively in mice receiving anti-E-selectin antibody (a) but not in mice receiving anti-VCAM-1 antibody (b). a and b, ×400.

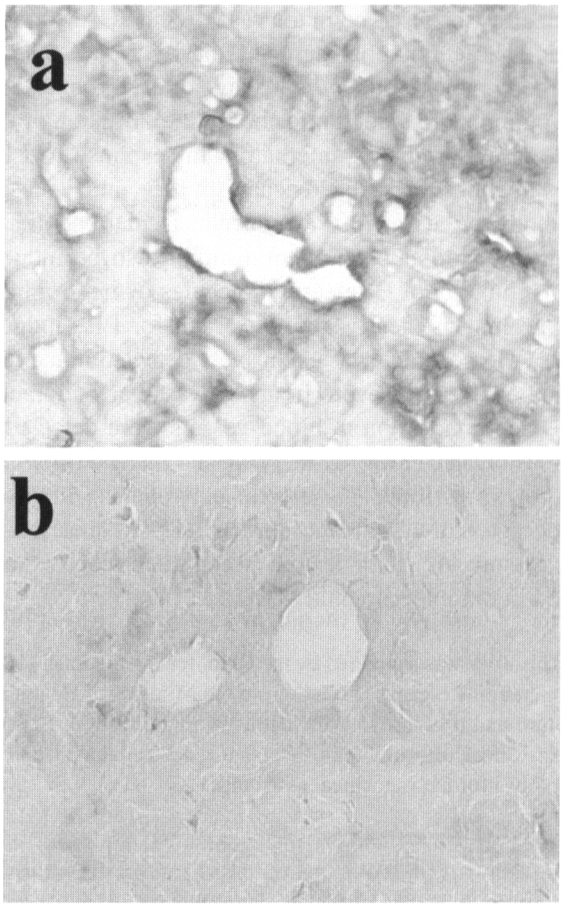

Fig. 3. The expression of E-selectin on the vascular endothelial cells of mice injected with LPS. The expression of E-selectin was immunohistochemically inspected in mice receiving a preparative injection of LPS (a) and untreated control mice (b). Positive staining was detected around the blood vessels in mice that received a preparative injection (a) but not in untreated control mice (b). a and b, ×400.

the administration of VCAM-1 antibody in place of anti-E-selectin antibody. It was therefore suggested that E-selectin might play a critical role in the exacerbation of LPS-induced vascular endothelial injury. This is the first report to determine that the interaction of E-selectin with anti-E-selectin antibody may exacerbate vascular endothelial injury in *in vivo* response to LPS.

The possibility that vascular endothelial injury was mediated by complement-dependent antibody-mediated cytotoxicity was excluded by several lines of evidence: first, the injection of the antibody alone did not result in vascular endothelial injury in E-selectin-positive LPS-sensitized mice; second, the injury of vascular endothelial cells was mediated with apoptotic cell death; third, anti-VCAM-1 antibody with the same Ig subclass did not induce such injury; and finally, no anaphylactic death occurring within a short duration was seen in mice injected with the antibody.

There are a number of reports on the inhibitory action of anti-E-selectin antibody on the binding of neutrophils to the endothelial layer (5, 6, 10, 17). Therefore, we speculated that anti-E-selectin or VCAM-1 antibody as well as anti-ICAM-1 antibody (12) might inhibit the binding of neutrophils to vascular endothelial cells and prevent vascular endothelial injury during GSR. In fact, Mulligan et al (15) reported that anti-E-selectin antibody markedly reduces neutrophil-mediated lung injury when IgG immune complex deposition was induced in the dermis and lungs of the rat. However, we found the exacerbation of vascular endothelial injury during GSR by the administration of anti-E-selectin antibody. Lorenzon et al (13) reported that anti-E-selectin antibody induced transient Ca influx and a rearrangement of endothelial cell cytoskeletal microfilaments. Moreover, Kaplanski et al (9) reported that the binding of E-selectin by specific antibody induces a marked rounding up and deformation of activated human endothelial cells, and no

such effect is observed in the case of anti-ICAM-1 antibody. The endothelial deformation by anti-E-selectin antibody may facilitate the leakage of erythrocytes into the pulmonary alveoli and enhance the diffusion of soluble proinflammatory mediators in response to LPS. This might be a possible mechanism how anti-E-selectin antibody exacerbated LPS-induced vascular endothelial injury. A similar phenomenon might also be induced by the binding of E-selectin molecules by neutrophils.

The lung is clearly a target organ for LPS-induced injury. Hemorrhage was exclusively detected in the lung lesions, although severe injury of vascular endothelial cells and congestion in blood vessels were detected in various organs. However, there was no marked difference in the expression of E-selectin between the lung and other organs of GSR-induced mice. It is still unclear as to the reason why lung lesions were selectively exacerbated in our experimental system.

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