Fc and C3b Receptors on Pulmonary Endothelial Cells: Induction by Injury

Abstract. *Receptors for the activated third component of complement and for the Fc portion of immunoglobulin G are not expressed by apparently normal bovine pulmonary endothelial cells, but are expressed when the cells are exposed to white cell lysates or are infected with influenza or cytomegalovirus. The unmasking of these latent receptors may contribute to the pulmonary inflammatory response characteristic of, for example, anaphylaxis and to those lung diseases characterized by the deposition of immune complexes.*

In previous studies, we have shown that healthy bovine pulmonary endothelial cells in monolayer culture do not exhibit receptors for the activated form of the third component of complement (C3b), or for the Fc portion of immunoglobulin G (IgG) (*1*). This finding led us to draw two inferences: (i) the lack of these receptors may contribute to the nonthrombogenic properties of endothelium; and (ii) although the lungs are a major target organ for immune complex deposition, the mechanism of binding of circulating immune complexes in the lungs is unlikely to be via endothelial C3b or Fc receptors. However, our previous studies were not designed to disclose latent receptors for C3b or Fc capable of expression as a consequence of injury to endothelial cells.

We have now examined the possibility that certain types of damage to the endothelium might induce or in some way trigger the expression of Fc and C3b receptors on the endothelial surface and render them more prone to attachment by circulating immune complexes. For example, certain viral infections appear to induce the expression of Fc receptors on a variety of cultured cells (*2*) and to induce granulocyte adherence to endothelial cells (*3*). Furthermore, severe disruption of endothelial cells resulting in exposure of intermediate filaments can lead to antibody-independent binding or trapping of complement components C1q, C4, and C3 (*4*). In view of the lung injury induced by leukocytic proteases (*5*), we postulated that proteases from, for example, polymorphonuclear neutrophilic leukocytes (PMN's) might effect injury of the pulmonary endothelium so as to induce the appearance of receptors not expressed by uninjured cells.

We compared bovine pulmonary endothelial cells from control cultures, cells that had been infected with influenza virus or cytomegalovirus, and cells that had been incubated with a bovine white cell (rich in PMN's) lysate (*6*) for their abilities to bind sheep erythrocytes (E), erythrocytes sensitized with IgG antibody (EA), or erythrocytes bound to C3b (EAC1423). Unsensitized erythrocytes and the complex EAC142 were used as the respective controls (*7*).

Bovine pulmonary artery endothelial cells were isolated and subcultured without exposure to proteolytic enzymes and were characterized as described previously (*8*). Confluent monolayers were infected with influenza virus or cytomegalovirus (*9*). The cells were used 4 days after infection, but before cytopathic effects were evident. Bovine leukocytes, isolated by dextran flotation (*6*), were prepared as follows: 27.2×10^6 bovine PMN's, together with a few mononuclear cells and lymphocytes, were subjected to one freeze-thaw cycle and then centrifuged. The supernatant fluid was passed through a sterilizing filter (0.45 μm).

Endothelial cells were incubated with the white cell lysate (diluted 1:5 in Hanks buffered saline solution) for 30 minutes at room temperature and then washed in Hanks solution. As in our previous study, the rosette method (*10*) was used to test for Fc receptors, and the immune adherence assay (*11*) was used to test for C3b receptors.

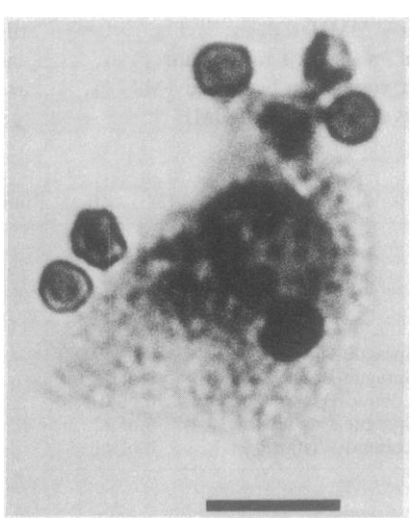

Fig. 1. Endothelial cells (~ 2×10^5) from the bovine pulmonary artery were treated with bovine PMN-rich lysate at room temperature for 30 minutes. The endothelial cells were washed with Hanks buffered saline solution and then incubated with 1 ml of EA (1×10^8 ml^{-1}) at 37°C for 15 minutes, then at 4°C for 2 hours. Rosettes (three or more attached cells) formed between endothelial cells and EA; 79 percent of the endothelial cells exhibited rosettes. The mean number of EA per endothelial cell was nine. Scale bar, 20 μm.

Both the EA and the EAC1423 were used at a concentration of 1×10^8 cells per milliliter. Bovine endothelial cells (passage 17) were used at a concentration of approximately 2×10^5 cells per milliliter. The endothelial cells were incubated with the red cell intermediate complexes for 15 minutes at 37°C. The cell suspensions were centrifuged gently (25*g*, 5 minutes, 4°C), and the incubation was continued for 2 hours at 4°C. The results were scored and photographed (Fig. 1) with a microscope (Zeiss Universal Research) fitted with a camera system (Olympus).

As before (*1*), control monolayers of bovine pulmonary endothelial cells did not exhibit receptors for Fc or C3b. However, cells exposed to the white cell lysate formed large rosettes with EA (Fig. 1) and to a lesser extent clumped EAC1423. The most striking induction of receptors was in response to the leukocyte lysate, although cells infected with cytomegalovirus gave positive, but less striking, results with EA and EAC1423. Influenza infection produced some rosette formation with EA, but no discernible immune adherence with EAC1423.

The most clear-cut and striking feature of the present findings was the induction of latent or otherwise unexpressed receptors for the C3b component of complement and for the Fc portion of IgG in endothelial cells that had been exposed to the white cell lysate. The strongest induction occurred when the endothelium, as a monolayer or as a suspension of cells, was exposed to the white cell lysate. Induction occurred without rupture of the endothelial cell membrane discernible by light microscopy. Our findings raise questions about the functional role of, for example, PMN proteases during leukocyte-induced lung injury. Ward (*12*) suggested that acute lung injury on the vascular (as opposed to airway) side can be triggered by the deposition of immune complexes in vascular walls or by intravascularly infused preformed immune complexes—effects that are both neutrophil- and complement-dependent. A similar vascular reaction is induced by infusion of C5a, a potent neutrophil-activating agent (*13*). Apparently, C5a can be activated in a wide variety of disease states and in many medical procedures, including cardiopulmonary bypass and renal dialysis (*14*).

The ability of lysates of PMN's and

0036-8075/81/1030-0557$01.00/0
Copyright © 1981 AAAS

two viruses capable of infecting endothelial cells to unmask receptors for the Fc fragment of IgG (and to a lesser extent for C3b) is likely to be of pathophysiologic importance. White blood cells activated by C5a or by immune complexes [as may occur in IgG aggregate anaphylaxis, chronic serum sickness, lupus erythematosus, and idiopathic pulmonary fibrosis (*12*)] marginate and attach to endothelial cells in vivo (*13*). Furthermore, endothelial cells infected with certain viruses have the capacity to bind PMN's, and the PMN's thus bound become adherent for other PMN's (*3*). White blood cell aggregates, such as those formed in anaphylaxis (*15*), might be expected to unmask Fc receptors on endothelial cells. The stagnant or reduced blood flow created by the mechanical blockage caused by white cell aggregates and emboli would provide favorable conditions for binding the Fc portion of aggregated IgG or immune complexes (both present in the plasma of anaphylactic subjects). In turn, endothelial cell–bound immune complexes would provide further stimulus for complement activation and complement-linked immune lysis. Presumably, this series of reactions would occur most prominently at the level of the microcirculation where mechanical blockage of blood flow would be most pronounced and where, in fact, the inflammatory response to, for example, anaphylaxis is most pronounced (*15*).

UNA S. RYAN
DUANE R. SCHULTZ
JAMES W. RYAN

Department of Medicine.
University of Miami
School of Medicine,
Miami, Florida 33101

References and Notes

1. U. S. Ryan, D. R. Schultz, P. J. Del Vecchio, J. W. Ryan, *Science* **208**, 748 (1980).
2. D. Westmoreland and J. F. Watkins, *J. Gen. Virol.* **24**, 167 (1974); M. Ogata and S. Shigeta, *Infect. Immun.* **26**, 770 (1979); A. P. Lyss, H. Friedman, R. Corky, N. Kefalides, M. Bina, S. T. Widirstky, D. B. Cines, *Circulation Suppl.* **62** (No. 3), 272 (1980).
3. R. R. MacGregor, H. M. Friedman, E. J. Macarak, N. A. Kefalides, *J. Clin. Invest.* **65**, 1469 (1980).
4. E. Linder, *J. Immunol.* **126**, 648 (1981); ———, V.-P. Lehto, S. Stenman, *Nature (London)* **278**, 176 (1979).
5. A. Janoff, R. White, H. Carp, S. Harel, R. Dearing, D. Lee, *Am. J. Pathol.* **97**, 111 (1979).
6. H. J. Fallon, E. Frei III, J. D. Davidson, J. S. Trier, D. Burk, *J. Lab. Clin. Med.* **59**, 779 (1962).
7. D. H. Vroon, D. R. Schultz, R. M. Zarco, *Immunochemistry* **7**, 43 (1970).
8. U. S. Ryan, E. Clements, D. Habliston, J. W. Ryan, *Tissue Cell* **10**, 535 (1978); U. S. Ryan, M. Mortara, C. Whitaker, *ibid.* **12**, 619 (1980); U. S. Ryan, in *Pulmonary Toxicology*, G. E. R. Hook, Ed. (Raven, New York, 1981).
9. Bovine pulmonary endothelial cells were infected with strains VR 95, lot 14D (influenza PR8), and VR 677, lot 3 (cytomegalovirus). The input multiplicity exceeded 1 plaque-forming unit per cell. Verification of infection of the endothelial cells with influenza virus was by hemabsorption with chicken erythrocytes, and with cytomegalovirus by cytopathology and inclusion body staining.
10. R. J. Winchester and G. Ross, in *Manual of Clinical Immunology*, N. R. Rose and H. Friedman, Eds. (American Society for Microbiology, Washington, D.C., 1976), pp. 64–76.
11. R. A. Nelson, J. Jensen, I. Gigli, N. Tamura, *Immunochemistry* **3**, 11 (1960).
12. P. A. Ward, *Am. J. Pathol.* **97**, 85 (1979).
13. P. M. Henson *et al.*, *ibid.*, p. 93.
14. D. E. Hammerschmidt, T. K. Bowers, C. J. Lammi-keefe, H. S. Jacob, P. R. Craddock, *Blood* **55**, 898 (1980).
15. M. L. Goodman, B. A. Way, J. W. Irwin, *Virchows. Arch. A* **383**, 271 (1979).
16. We thank J. Kern, American Type Culture Collection, for infecting endothelial cells with the virus strains and C. Whitaker, P. Arnold, J. Walling, and L. White for technical assistance. Supported by PHS grants HL 21568 and HL 22087, by the Council for Tobacco Research–U.S.A., Inc., and by the Kroc Foundation.

2 June 1981; revised 3 August 1981

Effect of Indomethacin on Intestinal Tumors Induced in Rats by the Acetate Derivative of Dimethylnitrosamine

Abstract. *Over the course of 20 weeks, Sprague-Dawley rats developed intestinal tumors in response to an intraperitoneal injection of the acetate derivative of dimethylnitrosamine. The same agent did not induce tumors in Lobund-Wistar rats. The number of tumors was significantly smaller in rats given drinking water containing indomethacin (beginning 14 days after the injections) than in control rats given drug-free water.*

It has been reported that DMN-OAc, the acetate derivative of dimethylnitrosamine, induces intestinal tumors in Sprague-Dawley rats after a single intraperitoneal injection (*1*, *2*). Sprague-Dawley rats were more susceptible to tumor induction than Fischer 344 and Buffalo strain rats and, among them, males were more susceptible than females. A dose-related induction of intestinal tumors in Sprague-Dawley rats was demonstrated 20 weeks after one, five, and ten doses of 1,2-dimethylhydrazine (DMH; 30 mg per kilogram of body weight) were administered by gavage at weekly intervals. In further experiments with the same protocol, DMH did not induce intestinal tumors in Lobund-Wistar rats; methylazoxymethanol acetate (MAM), the active metabolite of DMH, induced tumors in both strains (*3*). Subsequently it was demonstrated that the number of rats with tumors induced by five doses of DMH could be reduced significantly by oral administrations of indomethacin, a nonsteroidal anti-inflammatory drug that blocks the synthesis of prostaglandins (*4*). In Sprague-Dawley rats that received a single dose of DMH, the antitumor effect of indomethacin was even more significant (*4*). Indomethacin also had an antitumor effect in rats that had been inoculated with a single dose of MAM.

To determine the effect of indomethacin on tumors induced by another carcinogen, we gave male Sprague-Dawley and Lobund-Wistar weanlings single intraperitoneal injections of DMN-OAc (13 mg/kg). Both strains were from a closed

Table 1. Induction of intestinal tumors by DMN-OAc.

Rat strain	Rats with tumors	Tumors in colon	Tumors in small intestine	Mean number of tumors per rat
Sprague-Dawley	28 of 39	19	34	1.4
Sprague-Dawley	8 of 9	6	13	2.1
Lobund-Wistar	0 of 10	0	0	
Sprague-Dawley	6 of 12	1	7	0.66
Lobund-Wistar	0 of 11	0	0	

Table 2. Effect of indomethacin on induction of intestinal tumors by DMN-OAc.

Treatment	Rats with tumors	Tumors in colon	Tumors in small intestine	Mean number of tumors per rat
Indomethacin	1 of 7	0	1	0.14*
Control	6 of 8	5	7	1.5
Indomethacin	0 of 5	0	0	
Control	8 of 10	7	7	1.4

*$P < .05$, Student's t-test.

0036-8075/81/1030-0558$01.00/0 Copyright © 1981 AAAS