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Endothelial Cells of Bovine Pulmonary Artery Lack Receptors for C3b and for the Fc Portion of Immunoglobulin G

Abstract. *Bovine pulmonary endothelial cells do not possess receptors for the 3b component of complement (C3b) or for the Fc portion of immunoglobulin G. The lack of these receptors may help explain the nonthrombogenic function of endothelial cells. Our findings rule out the possibility that endothelial cells participate in pulmonary immune complex disease through the binding of C3b or Fc fragments.*

It is generally agreed that the lungs are a major target organ in some forms of immune complex disease (*1*). The lungs appear to be a major site of deposition of immune complexes in which antigen is in slight excess over antibody (*2, 3*). Our study was begun to help clarify the role, if any, of pulmonary endothelial cells in binding of immune complexes as they may occur within the circulation. Specifically, we examined for the presence of receptors for the Fc portion (crystallizable fragment) of IgG (immunoglobulin G) and for C3b (3b component of complement). We were interested in C3b both because of its role in immune adherence and immune conglutination (*4*) and because bovine serum contains conglutinin, which appears to have specificity for bound C3b. We have found that bovine pulmonary endothelial cells in culture (initial isolates through the ninth passage) do not possess receptors for C3b or the Fc portion of IgG.

We obtained the endothelial cells by scraping bovine pulmonary artery with a scalpel. The cells were dissociated in 0.1 percent collagenase and cultured in Medium 199 containing 20 percent fetal calf serum. We used methods similar to those described previously (*5*). The cells were identified by structural and functional criteria: monolayer growth with cobblestone appearance; ultrastructural characteristics of endothelial cells including frequent caveolae and occasional Weibel-Palade bodies (*5–7*); presence of angiotensin-converting enzyme activity (*6–10*); and reactivity with antibodies to human factor VIII and α_2-macroglobulin (*5*).

The rosette method (*11*) was used to assay for Fc receptors. Sheep erythrocytes (E), either alone or sensitized with IgG antibody (EA), were purchased from Cordis Laboratories. The sheep E and EA were washed three times and adjusted to a concentration of 0.5 percent in ice-cold Hanks buffered saline solution (HBSS) (*12*). To perform the assay, approximately 2×10^5 endothelial cells in 0.1 ml of HBSS were incubated with 0.1 ml of EA at 37°C for 15 minutes. The reaction mixture was then centrifuged very gently, at approximately 25*g* for 5 minutes, and incubated for an additional 2 hours at 4°C. The cells were resuspended and examined in a Zeiss photomicroscope with phase optics. Endothelial cells did not bind EA (Fig. 1a). Prior treatment and length of time in culture did not appear to be important factors. Fresh isolates were unreactive as were cells in monolayer culture (passages 4 through 9; tested 24 hours after passage). Cells detached mechanically by scraping or enzymatically (0.5 percent trypsin with EDTA) did not bind EA. The quality of the EA was verified by reaction with B lymphocytes, cells known to possess Fc receptors (Fig. 1b).

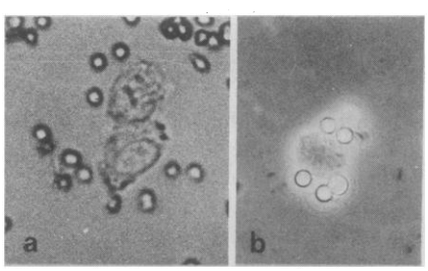

Fig. 1. (a) Cow pulmonary artery endothelial cells in the fifth passage were removed from their flasks with a rubber policeman. Approximately 2×10^5 endothelial cells were incubated with 3 ml of 0.5 percent EAC at 37°C for 15 minutes, then at 4°C for 2 hours. Endothelial cells did not bind EAC (×800). (b) Human buffy coat was incubated with 0.5 percent EAC as described for endothelial cells. Rosettes formed between B lymphocytes and EAC (×800).

The immune adherence assay (*13*) was used to test for C3b receptors. The cellular intermediate, EAC14b2a3b (EAC1423) was prepared as described (*14*). The cells were suspended in a dextrose-gelatin Veronal buffer containing 0.15 m*M* calcium and 0.5 m*M* magnesium, *p*H 7.3 (*13*), and adjusted to a concentration of 8×10^7 cells per milliliter. To confirm that C3b was bound to the EA, monospecific goat antibody to human C3b was added to the coated red cells and clumping occurred. This indicated that the cellular intermediate was EAC1423, as expected. Three dilutions of complement-bearing red cells, 4×10^7, 4×10^6, and 4×10^5 per milliliter, were used. Endothelial cells were used at a concentration of 5.5×10^5 per milliliter. For control tests we used human red cells (O positive) at 8×10^7 per milliliter. Pulmonary endothelial cells showed no agglutination when incubated with EAC1423. When the test was carried out under identical conditions with normal human red cells, clumping did not occur.

Since a primary function of endothelial cells is to provide a smooth, gliding surface for the passage of blood, it is not surprising that these cells do not possess receptors for C3b and the Fc portion of IgG. In particular, the properties of C3b immune adherence could be extremely disadvantageous for the normal functioning of endothelial cells. Nonetheless, recent results (*2, 3*) indicate that the soluble immune complexes can gain access to the interstitial space of the lungs. The route taken by the immune complexes from blood to interstitium is not yet evident. What is evident is that endothelial cells do not participate by way of receptors for C3b, nor do they possess receptors for the Fc portion of IgG, unlike epithelial cells of the kidney which do possess receptors for C3b (*15*). Our results do not rule out the possibility that other

0036-8075/80/0516-0748$00.50/0

components of complement [for example, C5a chemotactic factor (*16*)] may damage or otherwise interact with endothelial cells to induce intravascular thrombosis. We studied only endothelial cells of bovine pulmonary artery; it remains to be determined whether endothelial cells of other vascular beds or of other species do or do not possess receptors for C3b or the Fc portion of IgG. However, we believe that it is significant that Shin *et al.* (*17*), using human renal biopsy tissue, found that C3b-coated bacteria were bound by glomerular epithelial cells but not by endothelial cells.

Over the past 12 years it has become evident that endothelial cells have far more complex functions than previously envisioned. These cells, previously believed to be little more than a mechanical barrier between blood and parenchyma, possess peptidase enzymes, cyclooxygenase, phosphatase enzymes, protease inhibitors, hemostatic factors, and receptors for polypeptide hormones such as insulin and angiotensin II (*5, 6, 18*). Efforts to determine the subcellular distribution of these various components will be facilitated by the use of immunocytochemical techniques, which have already proved useful in locating angiotensin-converting enzyme (*5, 6*). We conclude that the absence of Fc receptors on pulmonary endothelial cells is of major practical importance. If, indeed, Fc receptors occurred in close juxtaposition to the components (antigens) of interest, one could not use specific IgG to locate them immunocytochemically without using Fab (antibody-binding fragments) or without adding rigorous controls to minimize nonspecific binding. Our results indicate that the binding of antibody to Fc receptors need not be a concern. Thus, it appears that the rate-limiting step in developing an improved understanding of the role of endothelium in hemostasis, the processing of hormones, and the transport of metabolites is that of obtaining the relevant monospecific antibodies.

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Nitrous Oxide from Soil Denitrification: Factors Controlling Its Biological Production

Abstract. *Increasing concentrations of nitrate, nitrite, and molecular oxygen enhanced production of nitrous oxide relative to molecular nitrogen during denitrification in soils. Soil acidity interacted with nitrate to increase the ratio of nitrous oxide to molecular nitrogen. In response to anoxic conditions, nitrous oxide production initially increased but nitrous oxide was then consumed, a pattern which resulted from the sequential synthesis of nitrogenous oxide reductases.*

The role of nitrous oxide (N_2O) in stratospheric chemical reactions (*1*) has led to concern that increased terrestrial production of N_2O may cause depletion of the earth's ozone shield (*2*). Nitrous oxide may also contribute to a warming of the earth's surface by influencing the radiative budget of the troposphere (*3*). As the amounts of industrially or biologically fixed nitrogen used for crop production increase, the quantity of N_2O produced by microbial nitrogen metabolism in soil may increase (*4*).

Nitrous oxide is a product of denitrification, but it is also produced during nitrification (*5*) and during reduction of NO_3^- to NH_4^+ (*6*). Denitrification in soil produces both N_2O and N_2; hence this bacterial process may serve either as a major source of N_2O or as a sink for N_2O, through the reduction of N_2O to N_2. Little is known about the environmental parameters that control the relative production of N_2O and N_2 during biological denitrification.

Using soils and bacterial isolates from soils, we have attempted to identify factors that control the proportion of N_2O and N_2 produced by denitrification. To focus on the biological control of N_2O evolution, we used well-mixed soil slurries, thus minimizing the influence of diffusion through the soil matrix. To directly and sensitively quantify N_2O and N_2 production, we added to the soils $^{13}NO_3^-$ produced at the Michigan State University cyclotron (*7*). We also used the inhibition of N_2O reduction by acetylene (*8*) coupled with gas chromatographic analysis of the N_2O, using thermal conductivity or ^{63}Ni electron-capture detectors (*9*). The ratio of N_2O to $(N_2O + N_2)$ can be determined by quantifying the N_2O produced in the presence and absence of acetylene (*9*). The data that we report here summarize the results from a Brookston loam soil. Similar results from other soils and bacterial cultures are described elsewhere (*10*).

To determine if N_2O is a free intermediate during the reduction of NO_3^- to N_2 in the heterogeneous denitrifying microflora of soils (*11*), we added $^{13}NO_3^-$ to two soils and three bacterial cultures, thus labeling the N_2O produced during denitrification (*7, 10, 12*). The exchange of the $[^{13}N]N_2O$ with pools of added nonlabeled N_2O ($^{14}N_2O$) was quantified after a 10-minute incubation in the Brookston soil (Fig. 1A). The labeled N_2O mixed rapidly and quantitatively with the nonlabeled N_2O pool in the soils and soil isolates (*10*). This freedom of exchange indicates that operationally N_2O exists as a

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