Virus Infection of Endothelial Cells Increases Granulocyte Adherence

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ABSTRACT Adherence of human granulocytes was measured on endothelial monolayers of human and bovine origin, grown in 35-mm Diam petri dishes and in cluster wells. Adherence to human endothelium in petri dishes using 1.0 ml of whole blood averaged 17.9±3.7%, and to bovine endothelium was 20.3 ±3.7%. Cluster wells required only 1/5 the endothelial cells needed for petri dishes, and 0.25 ml of whole blood yielded average adherence of 26.2±3.4 to human cells and 28.0±3.7 to bovine in the wells. The impact of infection of the endothelium by different viruses on subsequent granulocyte adherence was measured. Polio virus produced an acute lytic infection of human endothelial cells, with associated increased adherence to 185.4% of control 24 h after inoculation. Significantly increased adherence was noted at 6 h, before detectable cytopathic effect. Herpes simplex type I caused a similar rapidly lytic infection of bovine endothelium associated with increased adherence to 213.7% of control 6 h after inoculation. This augmented adherence could be demonstrated when granulocytes were suspended in physiologic saline solution, showing that antibody and complement need not be present. Trypsin treatment of infected monolayers did not prevent the augmentation, and supernate from infected monolayers increased the adherence of polymorphonuclear leukocytes to normal, uninfected monolayers. Chronic, slowly lytic infections, lasting 7 d or more, were induced with adenovirus in human endothelium and with measles virus in bovine cells. Adherence increased as virus was noted in the cell cultures on day 4, several days before cytotoxicity was seen. Thus, chronic viral infection of the endothelium appears possible, and results in increased granulocyte adherence. In naturally occurring disease, such an infection may act synergistically with adherent granulocytes to damage the endothelium, and may represent an in vitro model of vasculitis.

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INTRODUCTION

During the inflammatory reaction, circulating granulocytes adhere intensely to the endothelial surface as the first step in their movement out of the vascular compartment and into the site of inflammation (1, 2). Until recently the interaction of granulocytes and endothelial cells could only be studied in vivo by direct visualization of the microcirculation using tissue transillumination (2–4). The development of techniques for the cell culture of vascular endothelium (5–7) has created the opportunity to study the interaction of granulocyte suspensions and endothelial monolayers under controlled conditions in vitro (8–12). These investigations have demonstrated that granulocytes adhere more avidly to endothelial cells than to other cell types (8–10), that trypsinization of the endothelium inhibits granulocyte adherence (9), and that granulocytes are capable of damaging the endothelial cells when stimulated by complement (11, 12). Because of our interest in the early stages of inflammation, we have examined the effect of viral infection of endothelial monolayers on the subsequent adherence of granulocytes to them. Because virus infection can cause tissue damage, we theorized that increased adherence of granulocytes might be an early result of endothelial infection. We attempted to find an acute lytic infection of the endothelial cell, which might represent rapidly progressive destructive viral infections. We also sought a chronic, nonlytic infection that might affect adherence without disrupting the endothelial monolayer.

Work with human endothelial cells is hampered by

dependence on fresh umbilical cords necessary for primary cultures (5, 6). Because bovine aorta endothelial cells also will grow readily in primary cultures (7), we compared the adherence of human granulocytes to endothelial cells from both sources. We also modified the reported granulocyte adherence assay (9) so that smaller cell culture plates could be used. The new assay maintains the same accuracy and reproducibility as that reported with the larger culture plates, but requires only 20% as many endothelial cells per assay.

METHODS

Endothelial cultures. Human endothelial cell monolayers were established from cells obtained by collagenase treatment of human umbilical cord veins, by the method of Gimbrone et al. (6). The cells were grown in medium 199 as modified by Lewis et al. (13), supplemented with heat-inactivated 20% fetal calf serum (thus complement and antibody-free), and antibiotics (gentamicin, 50 μg/ml, and amphotericin B, 2.5 μg/ml). All endothelial cells were primary monolayers, 6–9 d of age, and had gone through three to five population doublings after isolation. All monolayers were near confluence at the time of viral infection.

Bovine calf aortas were obtained from a local abattoir within 1 h of death. Endothelial cells were obtained by collagenase treatment of these vessels, and isolated cells were cultured as above (7).

In the initial studies, monolayers were grown in 35-mm Diam plastic petri dishes, but the adherence assay was later adapted to 24-well tissue culture cluster plates (Costar Data Packaging, Cambridge, Mass.) that had a well diameter of 16 mm and a 2-ml fluid capacity. For scanning electron microscope (SEM)[1] studies, the monolayers were grown on 15 mm Diam round glass coverslips placed in the bottom of the cluster plate wells. Cells were fixed for SEM in 3% glutaraldehyde in 0.1 M cacodylate (pH 7.3). After fixation, cells were dehydrated in a graded ethanol series and critical point dried in a Denton DCP-1 dryer (Denton Vacuum Inc., Cherry Hill, N. J.). Specimens were sputter-coated with gold and viewed in a Philips 500 SEM (Philips Electronic Instruments, Inc. Mahwah, N. J.).

Granulocyte adherence assay. Granulocyte adherence (GA) to the endothelial monolayers grown in the 35-mm Diam petri dishes was measured as described (9); medium 199 was decanted from the cultures, and 1 ml of heparinized whole blood (5 U/ml) was added to triplicate plates, just covering the surface. Human blood was used in all experiments. The endothelial monolayer-blood overlay was incubated at 37°C and 100% humidity for 15 min without agitation, and then the blood was aspirated. Comparison of the granulocyte counts before and after incubation permitted determination of the percentage of GA to the endothelium. In some experiments, a pure suspension of granulocytes in Hanks' balanced salt solution (HBSS) was used rather than whole blood. Granulocytes were separated by Hypaque-Ficoll density gradient sedimentation (14), washed three times in modified Hanks' solution and suspended in HBSS at a concentration of $5–10 \times 10^6$ cells/ml. To determine whether viral-induced changes in adherence could be prevented by trypsin treatment of the endothelium, bovine endothelial monolayers infected with herpes virus 6 or 24 h earlier had their nutrient medium replaced by Earle's balanced salt solution containing 0.18% trypsin for a 10-min incubation at 37°C. The trypsin solution then was decanted, and GA measured by addition of heparinized whole blood as above. To determine whether infected monolayers released a factor that induced increased GA, bovine endothelial monolayers were infected with herpes virus and 24 h later the nutrient medium was collected, virus was removed by ultracentrifugation at 30,000 *g* for 1 h, and the medium was incubated with either fresh bovine monolayers or a preparation of pure polymorphonuclear leukocytes (PMN) before determination of GA. After normal endothelial monolayers were incubated with medium from infected cells for 4 h, it was decanted, the monolayers were washed once with HBSS, and a suspension of PMN in HBSS was added for determination of GA. Control monolayers were incubated with medium harvested from uninfected monolayers of the same age as the infected ones. In other experiments, a preparation of pure PMN was incubated for 30 min in medium from either infected or uninfected monolayers and then added to wells containing normal bovine endothelial monolayers for measurement of GA. All experiments were performed on at least three separate occasions, and the results reported represent the means±SE. In adapting the assay to cluster wells, it was found that 0.25 ml of blood gave analogous results to 1.0 ml in the larger dishes (Results), and so 0.25 ml was used in the cluster plate adherence assay. To determine whether adherence-modifying agents had the same effects as reported (9) when used in the cluster wells, atropine or propranolol was added to heparinized whole blood to a final concentration of 10 μM, incubated for 15 min at 37°C, and then assayed for percent adherence on endothelial monolayers in the cluster wells.

Viral techniques. A pool of the attenuated Chat strain of polio type 1 vaccine virus was prepared by passage in human embryonic lung cells (MRC-5). Herpes simplex type 1 virus was isolated from the trachea of a patient with pneumonia and a pool prepared in MRC-5 cells. Typing was performed in chick embryo fibroblast cells (15). Adenovirus type 7 was obtained from the Center for Disease Control, Atlanta, Ga., and a pool prepared in MRC-5 cells. Measles virus, prepared from the attenuated vaccine strain, Edmonston-Zagreb, was propagated first in chick embryo fibroblasts and then in MRC-5 cells.

After infection of endothelial cells, viral titrations were performed by pooling scraped cells with supernatant fluids from duplicate microtiter wells and inoculating serial 10-fold dilutions onto MRC-5 cells. Each growth curve of viral infection of endothelial cells represents the mean results of two separate inoculations, harvests, and titrations. Rhesus monkey kidney cells (Flow Laboratories, Inc., Rockville, Md.) inoculated with herpes simplex virus type 1 were harvested in a similar fashion to endothelial cells.

A direct herpes type 1 fluorescein conjugate was supplied by Dr. Edwin Lennette, Berkeley, Calif., and a direct fluorescent conjugate to measles virus was obtained from Flow Laboratories Inc., and to adenovirus from Microbiologic Associates, Walkersville, Md. The herpes and adenovirus conjugates were used at a 1:40 dilution, and the measles conjugate at a 1:10 dilution. At these working dilutions, there was intense fluorescence of infected endothelial cells and no fluorescence of uninfected cells. For immunofluorescence, cells were grown on 12-mm Diam glass cover slips, fixed in acetone at −20°C for 10 min, then incubated

[1] *Abbreviations used in this paper*: GA, granulocyte adherence; HBSS, Hanks' balanced salt solution; MOI, multiplicity of input; MRC-5, human embryonic lung (cells); PMN, polymorphonuclear leukocytes; SEM, scanning electron microscope; $TCID_{50}$, tissue culture infectious doses.

with conjugate for 30 min at 37°C, washed in phosphate-buffered saline and distilled water, and observed in a Leitz Ortholux II fluorescent microscope with an HBO 200 w/4 mercury lamp (E. Leitz, Inc., Rockleigh, N. J.).

Endothelial cells infected with virus were observed five times weekly for changes in cell morphology, using a Leitz inverted light microscope.

RESULTS

Studies of the assay system

Comparison of GA to human and bovine endothelial monolayers. GA to endothelial cells from both sources was compared in 35-mm petri dishes (Table I). Mean adherence of the human granulocytes to human endothelium was 17.9±3.7%, and to bovine endothelium was 20.3±3.7%. To confirm that modifications in GA known to occur on human endothelial monolayers (9) are also seen when bovine endothelial cells are used for adherence, whole blood was incubated with 10 μM atropine or propranolol and layered over bovine endothelial monolayers (Table I). Inhibition of adherence was noted with atropine and augmentation with propranolol, in a manner similar to that reported when human endothelial cells were used as the adhering surface (9).

Adherence in cluster plate wells. We grew bovine endothelial cells in 16-mm Diam wells, to determine whether granulocyte adherence could be measured accurately and reproducibly on this smaller surface. The 16 mm wells have a surface area of 201 mm^2 compared to 962 mm^2 for the 35-mm petri dishes (20.9% the area), allowing the investigator to prepare five times as many wells as petri dishes from the same number of endothelial cells. The wells have capacity for 2 ml of fluid, but the bottom of the well can be covered with a much smaller volume of blood. The percentage of GA is dependent on the volume of blood added for the 15-min incubation (Table II): with 1 ml of whole blood, mean GA was 7.8±4.2%, compared to 22.7±5.1% with 0.5 ml of blood, and 32.7±3.6% with 0.25 ml. Therefore, a volume of 0.25 ml of whole blood or granulocyte suspension was used in all subsequent experiments measuring GA in cluster plate well cultures. The whole blood GA to human and bovine cluster plate endothelial cultures was determined in five experiments: adherence to human monolayers averaged 26.2±3.4%, and to bovine 28.0 ±3.7%. The effect of agitation of the wells on GA of whole blood was assessed by gently swirling the blood in the wells for 5 s every 30 s during incubation, and comparing adherence on those monolayers with that on those left unagitated. Adherence to agitated monolayers was significantly reduced vs. unagitated monolayers ($P < 0.02$, Table III), and was almost as low as to wells containing no monolayers. However, the increase in GA to virally infected monolayers described below was seen to a similar degree with agitated and unagitated monolayers. Heparin concentrations between 0 and 25 U/ml had no significant effect on adherence of pure PMN in HBSS. At 50 U/ml there was significant inhibition of GA (control: 36.9±4.1% vs. 50 U: 28.8±2.4%, $P < 0.05$, t test).

TABLE I
Adherence of Human Granulocytes to Endothelial Monolayers of Human and Bovine Origin

	Human endothelium	Bovine endothelium
	%	%
Control	17.9±3.7 (6)*	20.3±3.7 (6)
Atropine (10 μM)	9.8±3.2‡ (3)	12.6±3.2 (4)
Propranolol (10 μM)	38.9±1.7‡ (3)	39.1±7.8 (4)

* Number of experiments.
‡ Reported previously (8).

TABLE II
Effect of Specimen Volume on GA to Bovine Endothelial Monolayers in Cluster Wells

	Volume of blood specimen assayed		
	1.0 ml	0.5 ml	0.25 ml
		%	
GA	7.8±4.2	22.7±5.1	32.7±3.6

Studies of GA on virus-infected endothelium

Acute, lytic infection of human endothelial cells by type I polio virus. Endothelial cells were infected with 500 tissue culture infectious doses ($TCID_{50}$) at a multiplicity of input (MOI) of 0.03. One $TCID_{50}$ is that amount of virus that will infect 50% of all inoculated cultures. MOI of 0.03 indicates that there are three infectious viruses for every 100 tissue culture cells. The titer of virus increased from 10^2 $TCID_{50}$ 3 h after inoculation to 10^4 at 24 h, and 10^6 at 48 h (Fig. 1). At 6 h after inoculation there was no cell damage evident by microscopy, but by 24 h, the monolayer was showing lysis, and at 48 h there was extensive cell destruction. GA to polio-infected cells increased to 135.5% of adherence to control monolayers 6 h after inoculation

TABLE III
Effect of Agitation of Monolayers on the Percentage of GA

	Normal monolayers	Herpes-infected monolayers	Significance of difference	Empty wells
Unagitated	13.7±1.4%	23.6±2.2	$P < 0.02$	4.6±0.5
Agitated	5.4±1.7%	15.9±0.7	$P < 0.01$	3.6±0.6

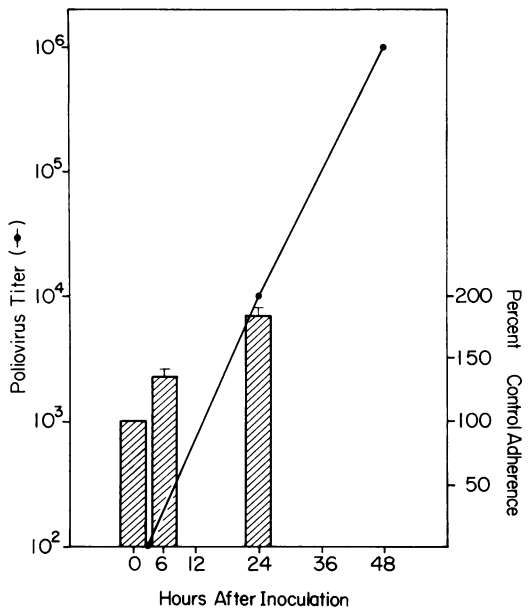

FIGURE 1 Poliovirus titer and granulocyte adherence after inoculation of human endothelial cells at a MOI of 0.03. Bars represent means of four experiments; brackets, ±SEM.

($P < 0.02$, paired sample t test), at a time when no cell damage was evident. GA increased to 185.4% of control by 24 h ($P < 0.001$), and by 30 h there was too much cell lysis for accurate determination of adherence.

Acute, lytic infection of bovine endothelial cells by type I herpes simplex virus. 10^3 $TCID_{50}$ of herpes simplex virus, type I, was inoculated onto bovine endothelial monolayers in microtiter wells at an MOI of 0.03 (Fig. 2). The titer was 0.5×10^1 3 h after inoculation, but by 24 h was 2.5×10^2; a maximum titer of 5×10^2 occurred at 48 h, and then was maintained at 10^1–10^2 for several days thereafter. A similar inoculum of virus added to the same volume of culture medium but without a cell monolayer had undetectable virus when assayed on days 1, 2, and 5 after inoculation. The same inoculum incubated on Rhesus monkey kidney cells did not yield detectable virus when sampled at the same intervals. Thus, the herpes virus titers found in the harvest from the endothelial cells after inoculation represent viral replication rather than persistence of the inoculum. Further evidence of viral replication was found when the monolayers were incubated with fluoresceinated antibody to the virus. The cells were negative for fluorescence 2 h after inoculation, 15% of the cells showed intense fluorescence at 24 and 48 h, and by 5 d there were areas of extensive cell destruction, with intense fluorescence in 15% of the remaining cells. GA increased to 213.7% of control at 6 h ($P < 0.05$, paired sample t test), was 166.7% at 24 h ($P < 0.01$), and 136.7% at 30 h ($P > 0.1$), a time when cytopathic effect was beginning to appear in the monolayer. Because the PMN adhering to the monolayer tended to obstruct a clear view of the cells, it was not possible to determine whether or not PMN were adhering preferentially to infected endothelial cells.

Lack of requirement for antibody and complement in increased GA to herpes-infected bovine endothelial monolayers. Granulocytes were separated from whole blood by Hypaque-Ficoll density gradient sedimentation, and suspended either in normal plasma, plasma heated to 56°C for 30 min before use, or in HBSS. Bovine endothelial cells were inoculated with 10^3 $TCID_{50}$ of type I herpes simplex virus, and GA was measured at 6 and 30 h after infection. Fig. 3 shows that adherence of granulocytes in all three suspending media was significantly increased above their adherence to uninfected cells at both 6 and 30 h after infection ($P < 0.05$, paired sample t test). Granulocytes suspended in HBSS had higher mean GA at 6 h than those in normal plasma or in heat-inactivated plasma, but the difference was not statistically significant. Thus, it appears that viral-induced increased GA does not require presence of complement or antibody.

Trypsin-insensitivity of increased GA induced by herpes virus. Bovine endothelial monolayers were infected with herpes virus, and GA of whole blood was measured 6 and 24 h later (Fig. 4). As in previous experiments (9), trypsin treatment significantly inhibited adherence to uninfected monolayers. However, an increase in GA was induced by virus infection that could still be detected after trypsinization, which was proportionally similar to the increase induced in

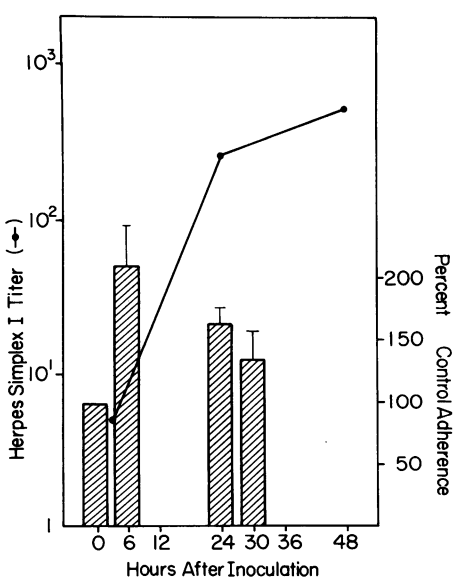

FIGURE 2 Herpes simplex I virus titer and GA after inoculation of bovine endothelial cells at an MOI of 0.03. Adherence could not be determined after 30 h because of cell destruction.

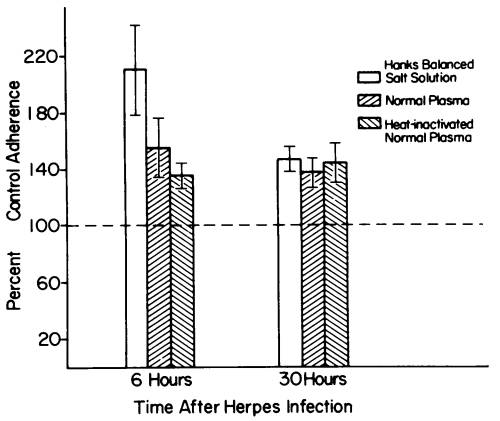

FIGURE 3 Effect of antibody and complement on GA to herpes-infected bovine endothelial cells. PMN were washed and resuspended in HBSS (devoid of antibody and complement), heat-inactivated pooled normal human plasma (lacking complement), and in normal plasma containing antibody and complement.

monolayers not exposed to trypsin. The increased adherence was significantly different from that to uninfected monolayers ($P < 0.02$, t test) both in monolayers trypsinized before GA measurement and in those not so treated. Thus, the hyperadherence induced by virus infection is not sensitive to trypsin treatment, unlike the intrinsic adherence found on uninfected monolayers.

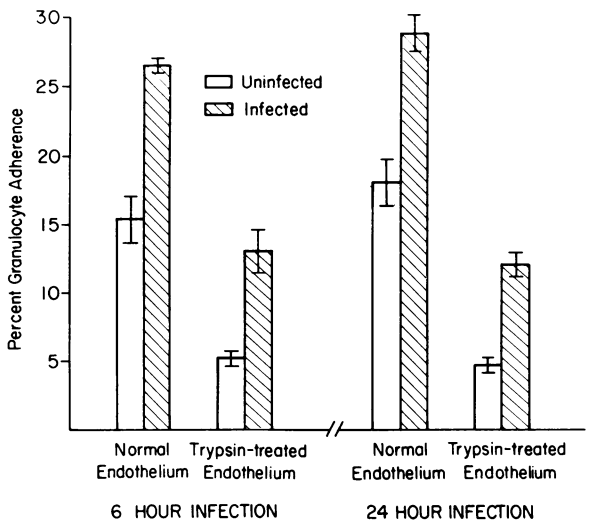

FIGURE 4 Effect of treatment of bovine endothelial monolayers with 0.18% trypsin on the increased GA induced by herpes simplex I virus infection. After either 6 or 24 h of infection trypsin treatment results in lower levels of adherence than on untrypsinized infected monolayers. However the increase in adherence in comparison to uninfected trypsinized monolayers was significant ($P < 0.02$) and proportional to the increases seen on untrypsinized infected monolayers.

Effect of supernate from infected and control endothelial monolayers on PMN and endothelial cells. When normal bovine endothelial monolayers were incubated for 4 h with supernate from herpes-infected endothelial monolayers there was no significant difference in GA to them vs. to monolayers incubated with supernate from uninfected monolayers (Fig. 5). In contrast, preincubation of PMN with supernate from infected cells caused a significant increase in their adherence vs. that of PMN incubated with supernate from uninfected endothelial monolayers ($P < 0.05$, t test). Thus, infected monolayers release a substance that acts to increase the intensity of PMN adherence but does not act directly on endothelial cells.

Chronic, slowly lytic infection of human endothelial cells by adenovirus. We looked for a less acutely lytic infection of endothelium, to enable more prolonged observation of adherence changes after inoculation. Adenovirus type 7 was able to cause a sustained infection of human endothelial cells (Fig. 6). Virus titers rose above inoculum concentration by day 4, and remained elevated through day 7. Lysis of the endothelial cells did occur, but at a rate much slower than with polio infection: no changes in cell morphology were noted until day 4, and actual lysis was not observed until day 7. GA remained unchanged until day 4, at which time it was found to be more than double the values found with uninfected control monolayers. By day 7 adherence had fallen to 137% of control values, concomitant with the development of endothelial cell lysis. Only the adherence changes noted on day 4 were statistically significant ($P < 0.05$, paired sample t test). The augmentation in adherence developed as the extent of infection increased: on day 2 when GA was still normal, only 5% of endothelial cells showed evidence of infection by fluorescent antibody staining. On day 4 when GA was significantly in-

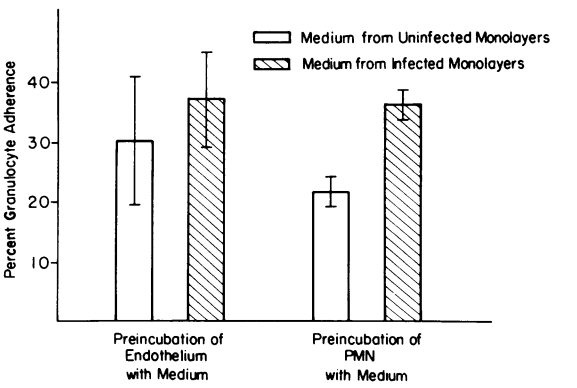

FIGURE 5 Incubation of bovine endothelial monolayers and PMN with medium from either normal endothelial cultures or from those infected with herpes simplex virus 24 h before.

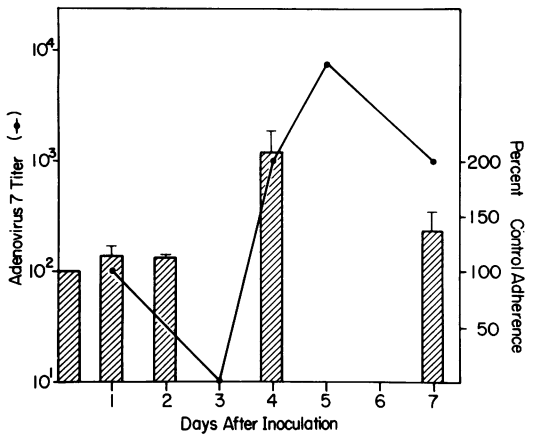

FIGURE 6 Adenovirus type 7 titer and granulocyte adherence after inoculation of bovine endothelial cells at an MOI of 0.3.

creased, 60–70% of the cells were positive for adenovirus by immunofluorescent staining.

Chronic, slowly lytic infection of bovine endothelial cells by measles virus. Inoculation of bovine endothelium with 10^4 $TCID_{50}$ measles virus at an MOI of 0.3 resulted in a very low-grade infection: titers were 0.5×10 2 h after inoculation, then increased to 5×10^1 at 1, 4, and 6 d, before falling to 5×10 at days 8 and 11 (Fig. 7). Little change in cell morphology was evident by inverted light microscopy until day 5, when syncytial cells begun to appear. Extensive lysis of the monolayer was evident on day 8. Intense nuclear staining characteristic of measles infection was detected by fluorescent antibody staining of the monolayer beginning 24 h after inoculation.

Using SEM, the measles-infected endothelial cells exhibit some minor changes in morphology when compared to controls 2 d after infection. Infected monolayers appeared to have larger intercellular spaces than did controls, and some cells bordering the spaces had lifted off the coverslip peripherally and appeared thickened or curled upward at the peripheral edge (Fig. 8a). Control cells are flat and squamous with central nuclei and rather uniform flat peripheries (Fig. 8b). On day 7 after infection, endothelial cells exhibited a grossly distorted morphology. While control cells existed as a sheetlike monolayer, the measles-infected cells were characterized by the formation of discrete clusters of cells with large gaps between the clusters. Individual cells within the clusters assumed a saucer-like configuration with curled edges (Fig. 8c). Control cells did not form the cell clusters found in the infected cultures (Fig. 8d).

GA was increased to a mean of 146.4% of control by 3 d after infection (Fig. 7), although this difference did not reach statistical significance ($P < 0.1$, >0.05, paired sample t test). On days 5 and 7 adherence was significantly increased ($P < 0.05$, paired sample t test), but the value on day 10 did not reach statistical significance, due to the small number of infected monolayers that survived that long to be assayed. By SEM, it can be seen that the virus-infected wells had more PMN adhering to them (Fig. 8). The PMN can be seen clustered on the endothelial cells and their extensions, avoiding the uncovered areas of the wells.

DISCUSSION

In 1977, Lackie and DeBono reported studies of the in vitro interaction of endothelial cells obtained from pig aortas with granulocytes from rabbit peritoneal exudates (8). They found that the granulocytes adhered better to endothelium than to fibroblasts and that the cells showed amoeboid movement over the monolayer. Unaware of their work, we investigated adherence of human PMN to endothelial monolayers derived from human umbilical veins, to determine whether the adherence phenomena that we had reported earlier with nylon fiber columns (16–19) would also be seen on endothelium (9). We noted a striking parallel between PMN adherence to nylon and to the endothelial monolayers, and found that adherence to fibroblasts, epithelial, and kidney cells was significantly less. Thus endothelial cells appear adapted for promoting adherence of PMN, which should aid the movement of PMN out of the intravascular compartment. This adherence-promoting characteristic could be completely destroyed by trypsin treatment of the endothelium, and the cells could replace the membrane components necessary for GA within 24 h of trypsin exposure. Hoover and colleagues showed

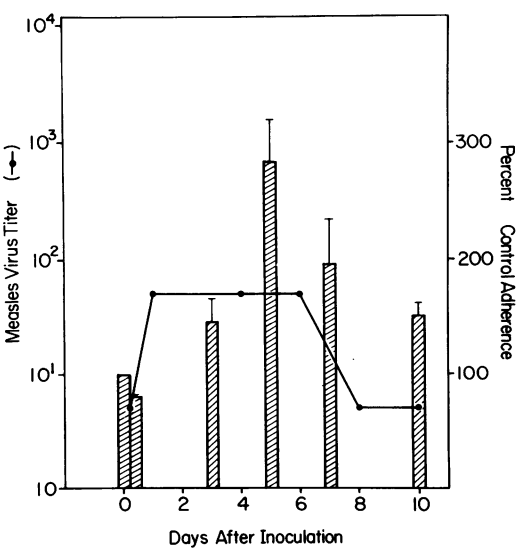

FIGURE 7 Measles virus titer and GA after inoculation of bovine endothelial cells at an MOI of 0.3.

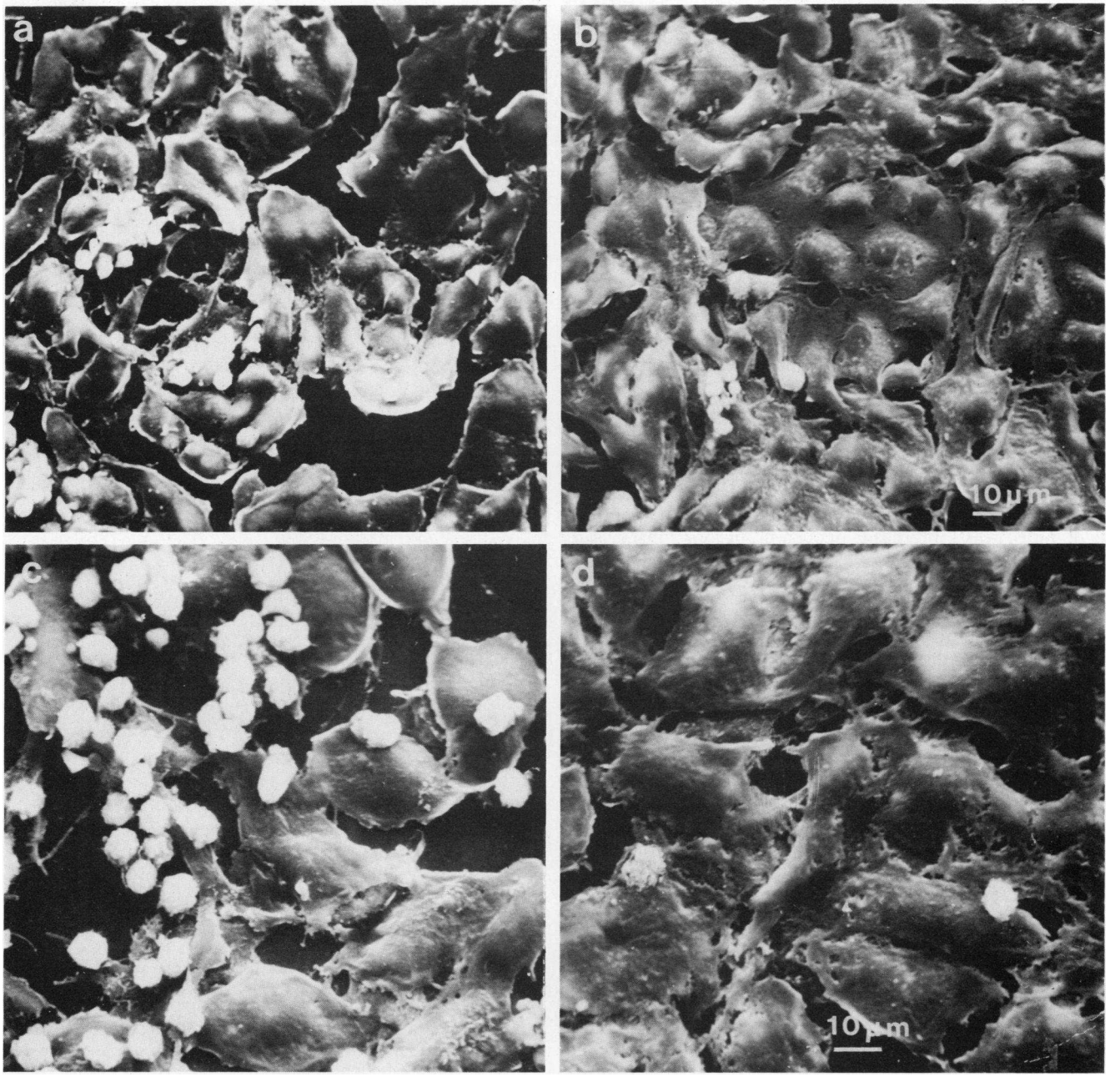

FIGURE 8 Bovine endothelial monolayers infected with measles virus. 8a shows cells on day 2 of infection, 8b shows uninfected cells of the same age. 8c shows cells on day 7 of infection, 8d uninfected controls. Note the GA to the cells and their extensions, greater for infected cells than uninfected controls of the same age. (a and b) are ×400. (c and d) ×800.

the same specificity of endothelial cells for GA, and found that PMN from both calf and human blood would adhere better to calf endothelium than to other cell types (10). They also showed that chemotactic factors enhanced adherence, as did treatment of the PMN with neuraminidase.

These three reports established that cell cultures of endothelial cells provide an excellent system for in vitro study of PMN-endothelium interactions. Because the cells are able to repair the trypsin-induced adherence defect in 24 h, we questioned whether some adherence-promoting membrane component normally present on the endothelium might be produced in increased quantities in response to injury or other

stimuli. Therefore, we chose virus infection as a probe, to see if it would modify endothelial-PMN interaction. It seemed reasonable that virus could infect endothelium in vitro, because viruses such as varicella and the pox viruses produce pathologic changes in the endothelium of skin vessels in vivo (20). Moreover, Andrews et al. have recently reported replication of human hemorrhagic fever viruses in endothelial cells (21).

We were interested in developing an acute lytic infection of both human and bovine endothelium, to study the effects of acute infectious injury on GA. We also sought a more subacute, nonlytic or slowly lytic infection, to determine whether more subtle injury would modify GA. Several important findings were noted in the present studies. First, adherence of human PMN to human and bovine endothelial cells was quite similar. Second, a cluster plate GA assay that conserved endothelial cells proved to be effective and reproducible. Third, increased GA to infected endothelium was noted with all viruses studied. Fourth, this increase occurred before observable cytopathologic changes in the infected monolayers by inverted light microscopy or SEM. Fifth, the increased GA did not appear to depend on the action of complement or antibody, as washed PMN suspended in HBSS also showed increased adherence to infected cells. Thus, although a reasonable mechanism for increased GA could be viral antigen reacting with antibody in whole blood to produce activated complement (which is known to increase GA [22]), changes can occur in a complement- and antibody-free milieu. Sixth, augmentation of GA to infected endothelium was not sensitive to trypsin, in contrast to normal PMN adherence, which is inhibited by trypsin treatment of the uninfected endothelium (9). Thus, it appears that the promotion of GA by viral infection is by a mechanism different from that responsible for normal adherence. Seventh, infection appears to stimulate the endothelium to release a soluble factor that acts directly on PMN to increase their adherence, in a manner similar to the factor that we have described in plasma of patients with acute inflammation (16).

The models of PMN-endothelial interaction that we report here have several implications. First, increased GA to endothelium induced by virus infections may cause the granulocytopenia reported with some viral diseases (23–25). Thus, increased adherence may cause a redistribution of PMN from the circulating granulocyte pool to the marginal pool, expressed as a fall in the clinical granulocyte count (which only measured the circulating pool [26]). The possibility that virus-induced granulocytopenia represents a redistribution rather than an actual loss of PMN could be tested by giving epinephrine to these patients with reduced counts, to determine whether their marginal pool is unusually large (27). A second intriguing implication of our findings is their possible relationship to clinical vasculitis. We have shown that a viral infection of the endothelium which is only slowly lytic can increase the adherence of PMN to it. Once these cells are adherent to the endothelium, they may diapedese, infiltrate the perivascular area, degenerate, and release lytic enzymes. Or, as has been shown by Sacks et al. (11) and Harlan et al. (12), PMN which are adhering to endothelium can be induced to damage these cells when exposed to activated complement components, endotoxin, or immune complexes. A study of the cytotoxic effects of PMN adherent to virally infected but nonlytic endothelial cells is needed. Increased PMN adherence to virally infected cells may be a general phenomenon; unpublished studies from our laboratory indicate that PMN adhere to fibroblasts infected by cytomegalovirus with much more intensity than to uninfected cells. Thus, it is possible that nonlytic viral infection of connective tissue may also induce an inflammatory reaction.

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