SUSCEPTIBILITY OF ENDOTHELIAL CELLS DERIVED FROM DIFFERENT BLOOD VESSELS TO COMMON VIRUSES

HARVEY M. FRIEDMAN, JAYNEANN WOLFE, NICHOLAS A. KEFALIDES, AND EDWARD J. MACARAK

Joseph Stokes Jr. Research Institute of the Children’s Hospital of Philadelphia (H. M. F., J. W.), Department of Medicine, University of Pennsylvania School of Medicine (H. M. F., N. A. K.), and Connective Tissue Research Institute, University of Pennsylvania (N. A. K., E. J. M.), Philadelphia, Pennsylvania

(Received 19 September 1985; accepted 17 January 1986)

SUMMARY

We examined whether endothelial cells derived from different blood vessels vary in their susceptibility to viral infection. Five common viral pathogens of humans (herpes simplex 1, measles, mumps, echo 9, and coxsackie B4 viruses) were evaluated for growth in endothelial cells derived from bovine fetal pulmonary artery, thoracic aorta, and vena cava. All five viruses replicated in each type of endothelial cell. There were apparent differences in the quantities of measles and mumps viruses produced in pulmonary artery endothelium compared with thoracic aorta and vena cava when endothelial cells were obtained from different animals. However, when pulmonary artery endothelial cells were compared with vena cava cells from the same animal, growth of each virus was similar in the two cell types. Four of the viruses replicated in the various endothelial cells without producing appreciable changes in cell morphology. These results indicate that endothelial cells from different blood vessels are equally susceptible to the human viruses evaluated, and that viral replication can occur without major alterations in cell morphology. Endothelial cells could serve as permissive cells permitting viruses to leave the circulation and initiate infection in adjacent tissues, including subendothelial smooth muscle cells.

Key words: endothelial cells; viruses; herpes simplex virus.

INTRODUCTION

Viruses have been implicated as possible causes of atherosclerosis (2,5,6,11,15,16), vasculitis (3,19), and proliferative lesions of the vascular endothelium (8-10). In a previous report, we demonstrated that many common human viruses replicate in vitro in endothelial cells derived from human umbilical vein or bovine thoracic aorta (2,11). Some viruses that were studied (polio and mumps) replicated only in human umbilical vein endothelial cells whereas another (coxsackie B4 virus) grew only in bovine thoracic aorta endothelium. The lack of growth of viruses in bovine endothelium was possibly related to the inability of certain viruses, which are human pathogens, to infect across species barriers. However, the possibility also existed that endothelial cells derived from different vessels vary in their susceptibility to virus infection. To evaluate this, we grew endothelial cells from different major vessels of a single species (bovine) and examined them for viral replication.

MATERIALS AND METHODS

Cell cultures. Primary cultures of bovine fetal pulmonary artery, thoracic aorta, and vena cava endothelial cells were established as previously described (14). The chest of the animal was opened to expose the thoracic cavity, the vena cava was severed below the diaphragm, and the entire heart and lungs were removed and placed in a container filled with chilled (medium 199) supplemented with amphotericin B (2.5 μg/ml) and gentamicin (50 μg/ml). Vessels were transported to the laboratory on ice and the remainder of the isolation carried out in a laminar flow hood. Because of their small size, vessels were clamped at either end with hemostats rather than sponge clamps. All other procedures were carried out as previously described (14). Cells were characterized as endothelium by their morphology and by demonstrating Factor VIII-von Willebrand protein by fluorescence microscopy. In earlier studies, different animals served as the sources for pulmonary artery, aorta, or vena cava endothelial cells. In later experiments, comparisons of viral replication in the various types of endothelial cells were performed on vessels from the same animal.

Viral infection. The sources of herpes simplex virus type 1 (HSV 1, NS strain), measles virus, mumps virus, coxsackie B4 virus, and ECHO-9 virus were as previously described (7). Endothelial cells were grown in 24-well
microtiter plates and infected at a ratio of one infectious virus per cell for experiments using HSV, ECHO-9, and coxsackie B4; or at a ratio of 0.1 infectious virus per cell for measles and mumps viruses. At various times postinfection, cells and supernatant fluids were harvested for viral titrations (7). For some experiments, viral antigens were detected in infected endothelial cultures by immunofluorescence using the following reagents: fluorescein-conjugated rabbit antimeasles serum at a 1:10 dilution (Flow Laboratories, McLean, VA); fluorescein-conjugated guinea-pig antimumps serum at a 1:10 dilution (MA Bioproducts, Walkersville, MD); horse anticoxsackie B4 serum at a 1:10 dilution (National Institute of Health, Bethesda, MD); and fluorescein-conjugated goat antihorse IgG at a 1:10 dilution (Cappel Laboratories, Downingtown, PA).

RESULTS

Viral replication. HSV 1, echo type 9, coxsackie B4 mumps, and measles viruses all replicated within endothelial cells derived from each of the vascular tissues examined. Figure 1 shows the growth curves of these viruses in each of the cell strains. For three viruses (HSV 1, measles, and mumps), replication was unequivocal in that amount of virus produced during the later times after infection exceeded the amount detected 2 h postinoculation (Fig. 1 a, d, e). However, for the enteroviruses (echo 9 and coxsackie B4) the maximum viral titers on Days 1 through 9 postinoculation did not appreciably differ from the titers of virus detected at the first sampling 2 h postinoculation (Fig. 1 b, c). To distinguish between survival of virus present in the inoculum and viral replication, echo 9 and coxsackie B4 viruses were inoculated into 24-well culture plates which contained medium but no monolayer of cells. In the medium alone, viral titers declined rapidly when incubated at 37°C (Fig. 1 b, c). This indicates that the virus detected after inoculation of the endothelial cultures was a reflection of viral replication and not merely survival of input virus. For confirmation, immunofluorescence studies were performed after inoculation of pulmonary artery and thoracic aorta endothelial cells with coxsackie B4 virus. In both cell strains, viral antigens were not detected on Day 1 postinoculation, but were present on Days 4 and 7.

For measles and mumps viruses, the peak viral titers obtained in pulmonary artery endothelial cells were higher than those obtained in vena cava or thoracic aorta cultures (Fig. 1 d, e). To examine these differences in more detail, comparative studies were performed on endothelial cultures derived from the pulmonary artery and the vena cava of the same animal. The two cell types were infected at the same time and harvests prepared for viral titrations and immunofluorescence at similar intervals postinoculation. Under these conditions, no differences were detected in viral replication (Fig. 2 a, b). In addition, by immunofluorescence the percentage of pulmonary artery and vena cava endothelial cells which

---

**FIG. 1.** Viral growth curves in bovine pulmonary artery ■-■; thoracic aorta △- △; and vena cava ○-○ endothelial cell cultures. +-- + Survival of virus in cultures containing media but no endothelial cell monolayer. Each curve is the mean of two or three separate infections, except for mumps virus infection of vena cava cells which was performed once.