Membrane related effects in endothelial cells induced by human cytomegalovirus

A. G. M. van Geelen¹, M. E. P. Slobbe-van Drunen¹, A. D. Muller², C. A. Bruggeman¹, and M. C. E. Van Dam-Mieras³

¹Departments of Medical Microbiology and ²Department of Internal Medicine, University Hospital of Maastricht, Maastricht, ³Department of Natural Sciences, Open University Heerlen, Heerlen, The Netherlands

Accepted May 2, 1995

Summary. Previously, we have reported on the increase in procoagulant activity of human umbilical vein endothelial cells (HUVEC) after infection with human cytomegalovirus (HCMV). When using microvascular endothelial cells from foreskin (MVEC), we also observe a significant increase in membrane perturbation and a concomittant increase in procoagulant activity. This effect is both observed with a laboratory HCMV strain (AD169) with low pathogenicity for endothelium and a HUVEC adapted strain (VHL-E) that readily infects endothelial cells. We compared the membrane perturbation of two types of endothelial cells, HUVEC and MVEC with human embryonal fibroblasts (HEF), being fully permissive for both strains. A membrane effect was only found in endothelial cells. Our results suggest that HCMV induces in MVEC more merocyanine-540 incorporation in the membrane as in HUVEC. The increase in the procoagulant activity induced by HCMV was more pronounced in MVEC than in HUVEC. Inactivated virus, as well as virus pre-incubated with heparin was unable to evoke membrane perturbation. It therefore appears that HCMV induces a rapid membrane response in vascular endothelium and that physical interaction of the virion and the endothelial cell is required to elicit this response.

Introduction

Human cytomegalovirus (HCMV), an ubiquitous pathogen in man, is a major mortality factor in immunocompromised individuals, such as AIDS-patients [12, 14, 17, 22], organ transplant recipients [2, 9, 16, 56] and newborn infants [13, 30]. Like other herpesviruses [27, 32, 36, 41, 49], HCMV can establish a life-long latency after initial infection. The site of latency [21, 42, 4] and the mechanisms by which reactivation will occur are presently not understood. In immunodeficient patients, the virus reactivates, posing a life-threatening risk.
HCMV has also been suggested to be a contributing factor in vascular diseases like transplantation associated arteriosclerosis [5, 20, 34]. These indications have been obtained through epidemiological [20] and pathological [21, 35] studies and by work in animal models [28, 29]. Putative mechanisms by which HCMV contributes to chronic vascular disease are largely unknown still, but in acute HCMV infections, especially in immunosuppressed patients, the virus is detected in the microvascular endothelium of most organs [37, 40, 43].

The endothelium is an important mediator of the cellular immune response both directly through antigen presentation [8, 53] and indirectly by actively contributing to the rolling [25, 38] and attachment [26] of leukocytes. We and others have reported on the alteration of several of these functions through HCMV infection of endothelial cells. Studies in our laboratory have shown that infection of endothelial cells with HCMV resulted in a marked increase in adherence of granulocytes and monocytes to endothelium while no increase in platelet adhesion was observed [44–46]. In addition, HCMV infection leads to enhanced expression of the adhesion molecules ICAM-1 and E-selectin [5] and it also increases the procoagulant response [51]. This procoagulant response is probably the result of facilitated interaction of coagulation factors on the perturbed surface of infected endothelial cells.

The interaction of HCMV with endothelial cells therefore provides a challenging focus of research.

We and others [6, 15, 23, 54] have reported on the limited infection rate of human umbilical vein endothelial cells (HUVEC) with HCMV laboratory strains. Dependent on the amount of viral input (MOI), up to 10% of the cells contain viral antigens with a concomitant disappearance of von Willebrand factor in the infected cells [7].

In addition to HCMV strain variation, also the origin and type of endothelial cells [31, 39] could be a determining factor in their responsiveness to HCMV infection. HUVEC are a frequently employed model for the study of endothelial cell function, but they appear to resemble the endothelium of the larger vessels, rather than that of the microcirculation where in vivo the virus is thought to exert its effect [3, 37]. In this report we describe the influence of HCMV infection on the procoagulant response and membrane perturbation in microvascular endothelial cells (MVEC), derived from human foreskin, and a comparison is made with HUVEC and human embryonal fibroblasts (HEF).

**Materials and methods**

**Cells and virus**

Human embryonal fibroblasts (HEF, Flow 2002) were cultured in Earl’s Modified Eagle Medium (EMEM), supplemented with 10% Newborn Calf serum and 50 μg.ml⁻¹ gentamicin. Cells between passage number 20 to 25 were used for experiments. HUVEC were harvested from umbilical cords as previously described [24]. HUVEC were cultured in plastic wells coated with fibronectin (10 μg/cm²) in growth medium consisting of 40% M199, 40% RPMI-1640, 10% pooled human serum (HCMV negative), 10% Fetal Bovine Serum,