The role of interferon \( \beta \) in human cytomegalovirus-mediated inhibition of HLA DR induction on endothelial cells

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Summary. Human cytomegalovirus (HCMV), a member of the virus family Herpesviridae that is associated with extensive worldwide morbidity and mortality in immunocompromised hosts, inhibits interferon-\( \gamma \) (IFN\( \gamma \))-mediated induction of human leukocyte antigen (HLA) class II antigens on endothelial cells. In this study, the ability of HCMV-infected endothelial cells to synthesize interferon-\( \beta \) (IFN\( \beta \)), and the role of IFN\( \beta \) in HCMV-mediated inhibition of HLA class II induction, was investigated. As determined by an encephalomyocarditis virus protection assay, HCMV-infected endothelial cell culture supernatants contained 240 IU/ml of IFN type I activity, of which 99.9\% was IFN\( \beta \), as compared to the absence of IFN\( \beta \) in mock-infected culture supernatants. UV-irradiated supernatants from HCMV-infected cultures inhibited induction of HLA class II in noninfected cultures by 24\%. This inhibition could be abolished with 500 NU/ml of anti-IFN\( \beta \) antibody. Addition of anti-IFN\( \beta \) antibody directly to HCMV-infected cultures mitigated but did not abolish HLA class II antigen inhibition. Dual immunohistochemistry for HCMV and HLA DR demonstrated that infected cells, in contrast to noninfected cells, were rarely induced to express HLA class II even in the presence of anti-IFN\( \beta \) antibody. These findings suggest that HCMV inhibits induction of HLA class II antigens by IFN\( \beta \) dependent and independent mechanisms.

Introduction

Infection with human cytomegalovirus (HCMV), a member of the virus family Herpesviridae, subfamily Betaherpesvirinae, is associated with severe morbidity in individuals with immature or immunosuppressed cellular immune systems. Many of these infections result from dissemination of virus which has persisted from a prior infection [17, 22].

One mechanism by which HCMV may develop persistence following primary infection is through inhibition of host cell human leukocyte antigen...
(HLA) expression with resultant escape from normal antiviral immune surveillance [33, 34]. Major histocompatibility (MHC) class I molecules, in associated with viral peptides, appear to represent the principal target for cell-mediated antiviral responses; however, MHC class II molecules may also function in the response to viral infections [5]. Viral antigens can be presented in the context of MHC class II molecules and be recognized by MHC class II-restricted cytotoxic T cells [1, 19, 26, 31]. Thus, virus-induced reductions in HLA class II expression may contribute to persistence in a manner analogous to that reported for murine lymphocytic choriomeningitis virus (MLCMV) infections, in which infected neurons do not express MHC class I and are resistant to MLCMV-specific T cell mediated cytolysis [21].

Potential sites of HCMV persistence include endothelial cells (ECs), which have been shown to be targets of infection in vivo [32, 42], and which can be readily infected in vitro [16, 45]. ECs possess a diverse array of immunologic functions including expression of HLA class II molecules in response to IFN\(\gamma\) [35, 36]. ECs expressing HLA class II molecules are capable of presenting antigens and can be specifically lysed by cytotoxic CD4 + T lymphocytes [15, 35]. Scholz et al. [40] reported that endothelial cell cultures, with a 50% level of HCMV infection, exhibited reduced expression of HLA class II antigens in response to IFN\(\gamma\). We have observed that individual human endothelial cells infected with HCMV fail to express interferon-\(\gamma\) (IFN\(\gamma\))-induced surface and cytoplasmic expression of HLA class II molecules, which is associated with absence of HLA class II mRNA [41]. Although this inhibition principally occurs within infected cells, it also affects expression within noninfected ECs within the cultures. The mechanisms by which HCMV infection of endothelial cell cultures results in inhibition of HLA class II induction within both infected and noninfected endothelial cells is unknown.

HCMV-mediated inhibition of HLA class II expression in ECs may be secondary to induction of type I interferons, interferon-alpha (IFN\(\alpha\)) and IFN\(\beta\) [10, 14]. These proteins have a wide range of antiviral activities which include stimulation of natural killer cell function and upregulation of MHC class I expression (reviewed in [3]). Type I IFNs have also been shown to interfere with IFN\(\gamma\)-mediated induction of MHC class II expression in murine macrophages and in human endothelial cells [18, 25, 27].

In addition to RNA viruses and poly(I):poly(C), DNA viruses are capable of inducing type I interferon synthesis. HCMV, which has a large, linear double-stranded DNA molecule, induces IFN\(\beta\) synthesis in fibroblasts eight to 16 h post-infection [2, 38]. There are no reports that human ECs produce IFN\(\beta\) or IFN\(\alpha\) following infection with HCMV [44]; however, human ECs are capable of synthesizing IFN\(\beta\) in response to infection with Newcastle disease virus, Sendai virus, vesicular stomatitis virus and poly(I):poly(C) [11].

Herein, we have examined the role of IFN\(\beta\) in the phenomenon of HCMV inhibition of HLA class II induction utilizing human umbilical vein endothelial cells (HUVECs), blocking antibodies specific for IFN\(\beta\), flow cytometry, dual-label immunohistochemistry, and HCMV isolate VHL/E, which exhibits