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Induction of class II molecules by cytomegalovirus in rat heart endothelial cells is inhibited by ganciclovir

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Abstract Cytomegalovirus (CMV) has been demonstrated to induce class II antigen expression in endothelial cells. To study whether ganciclovir (DHPG) has an effect on CMV-induced class II expression, cultured rat heart endothelial cells were infected with rat CMV (RCMV) and treated with different DHPG concentrations. Class II antigens in endothelial cells were detected by a monoclonal antibody and immunoperoxidase technique. Control cells did not express class II antigen, but during RCMV infection 92% of cells were class II-positive. DHPG treatment (1, 10, 100 and 1000 μg/ml) decreased RCMV-induced class II expression from 73% to 59%, 6% and 0%, respectively. As DHPG inhibits CMV DNA polymerase, our present results suggest that DHPG affects RCMV-induced class II expression via the inhibition of RCMV DNA replication.

Key words Cytomegalovirus
Ganciclovir · Endothelial cells
Class II antigens

Introduction

An association between cytomegalovirus (CMV) infection, heart allograft rejection and atherosclerosis has been described [1]. It has also been demonstrated that CMV induces MHC class II antigen expression in endothelial cells in vivo and in vitro [2]. Ganciclovir (DHPG) is a potent drug inhibiting the DNA synthesis of CMV, and it is therapeutically effective against CMV disease. DHPG has been used in the treatment of serious CMV infections in patients with immunodeficiencies in doses of 5 mg/kg body weight [3]. In this study we have investigated the rat CMV (RCMV)-induced MHC class II expression in cultured rat heart endothelial cells and the inhibitory effect of DHPG.

Materials and methods

Endothelial cells were isolated enzymatically from the hearts of 4–7 day-old DA rats [2]. The cells were cultured and characterized by an anti-FVIII antibody and immunofluorescence (IF). After the second passage, cells were infected with two concentrations (0.01 and 0.001 multiplicity of infection, MOI) of RCMV (strain RB61). The cultures were incubated until a cytopathic effect (CPE) occurred. In parallel, another set of cultures inoculated with RCMV were supplemented with DHPG at various concentrations (1–1000 μg/ml) and incubated equally long as the untreated infected cells. Normal rat heart endothelial cells were used as controls. The infection was confirmed by a monoclonal antibody against RCMV antigens and TF staining. MHC class II antigens were demonstrated by a monoclonal antibody (Mas 043 s, Sera-Lab) and immunoperoxidase staining.

Results

CPE occurred in the cultures of both RCMV concentrations after 4–10 days of incubation. Class II expression was recorded on 92% of RCMV-infected cells without DHPG. DHPG inhibited the induction of class II antigen but the inhibitory effect was dependent on the DHPG and RCMV concentrations. The frequency of class II-positive cells decreased with increasing concentrations of DHPG (1, 10, 100 and 1000 μg/ml) to 73%, 59%, 6% and 0% at 0.01 MOI of CMV, respectively. Also, the CPE of RCMV infection was totally inhibited with 1000 μg/ml of DHPG. At the lower concentration of RCMV (0.001 MOI), even 50 μg/ml of DHPG totally inhibited RCMV infection and the induction of class II antigen. Non-infected control rat heart endothelial cell cultures remained class II negative.

Discussion

We have described here the ability of DHPG to inhibit RCMV-induced class II antigen expression in leucocyte-free rat heart endothelial cell cultures. Because DHPG is an effective inhibitor of RCMV DNA polymerase, our results indicate that RCMV-induced class II expression is dependent on viral DNA synthesis. Although it is generally accepted that the gamma-interferon produced by activated T lymphocytes induces class II antigen expression, other pathways may also exist via different cytokines or their combinations. Our results suggest that DHPG may potentially inhibit the RCMV-induced class II antigen expression in vascular endothelial cells in heart allografts after transplantation and block the inflammatory cascades leading to heart allograft arteriosclerosis.

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