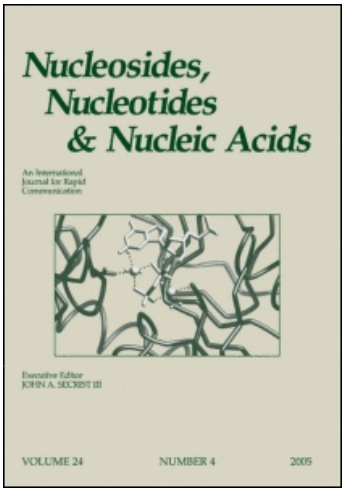

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ANTISENSE OLIGONUCLEOTIDE ISIS 2922 TARGETS IE-EXPRESSION AND PREVENTS HCMV-IE-INDUCED SUPPRESSION OF TSP-1 AND TSP-2 EXPRESSION

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ABSTRACT

ISIS 2922, but not ganciclovir (GCV), inhibits HCMV immediate early protein (IE) expression in different infected cell lines and prevents down-modulation of extracellular matrix proteins thrombospondin-1 and -2 induced by IE proteins. While action of ISIS 2922 is mainly due to specific inhibition of IE 2 mRNA, there is also evidence for unspecific effects in terms of inhibition of virus adhesion and penetration.

Human cytomegalovirus (HCMV) pathogenesis may be due to changes of cellular gene expression caused at least in part by HCMV immediate early proteins (IE) (1–7). Targeting of IE by antisense approach represents a treatment strategy to prevent both virus replication (9,10) and alteration of cellular gene expression (2,6–7). Effects of HCMV IE mRNA inhibitor ISIS 2922, a phosphorothioate antisense oligonucleotide, was studied in different cell types.

Human retinal glial cells (HRG) and human foreskin fibroblasts (HFF) cultured in vitro were infected with HCMV AD169 and Hi91, respectively. Mock-infected cultures served as controls. Cells were cultured in the presence of ISIS 2922, a non-complementary homologue (ISIS 26062) and anti-CMV nucleoside analogue ganciclovir (GCV). ISIS oligonucleotides were kindly provided by Isis

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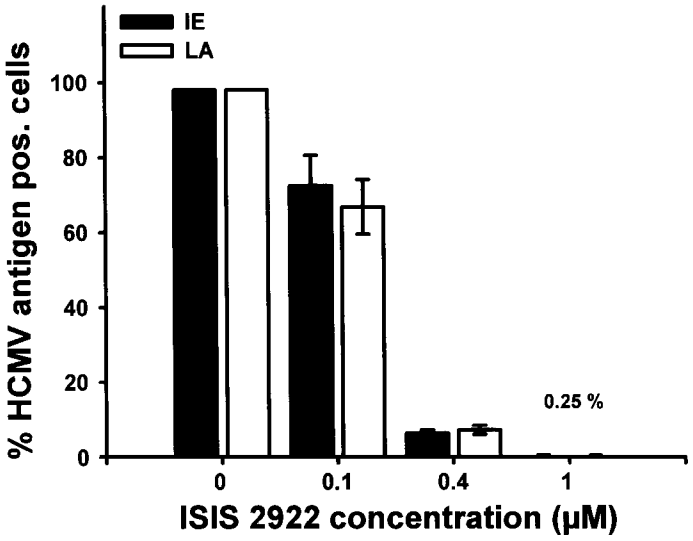

Figure 1. Effects of ISIS 2922 on IE and LA expression in HFF cells infected with M.O.I. 1-2.

Pharmaceuticals, Inc. (Carlsbad, Ca). For treatment, ISIS oligonucleotides were complexed with cationic liposomes DOTAP (Roche, Mannheim, Germany) according to the instructions given by the producer. Cells were preincubated with DOTAP/ISIS 2922 complex overnight in IMDM supplemented with 0.2% FBS (7,9,10). Before infection, cells were washed 3 times with PBS and virus was added for 1 hr adsorption time. After adsorption, cells were washed and DOTAP/ISIS 2922 complex was added again. Cells were stained for IE after 24 hrs and late protein after 72 hrs.

To detect unspecific blocking of virus entry, HFF cells were preincubated in a 6 well plate with ISIS 2922 and DOTAP mixture overnight, washed 3 times with PBS and infected with a extended virus adsorption time of 4 hrs at a MOI of 1-2. Inhibitor of virus-replication ganciclovir (40 μM, Hoffman-la Roche AG, Grenzach-Wyhlen, Germany) and inhibitor of virus binding dextrane sulfate (10 μg/ml) were added during virus adsorption. Cells were washed 3 times with PBS to remove unbound virus and disturbed by freezing and thawing. Intracellular amount of virus particles was measured as HCMV DNA copies/ml by quantitative RT-PCR (Roche, Mannheim, Germany). Extracellular matrix glycoprotein TSP-1 (3–6) and -2 mRNA level was measured by semi-quantitative RT-PCR (3). Indirect immunofluorescent detection of cell associated TSP-1 and -2 proteins was carried out using specific mouse monoclonal antibodies and fluorescein isothiocyanate conjugated goat anti-mouse IgG as secondary antibodies (Becton Dickinson, CA). Antibodies to IgG were used as control. Fluorescence was determined using Becton Dickinson FACScan and CellQuest software (Becton Dickinson, CA). All experiment were repeated at least 3 times.

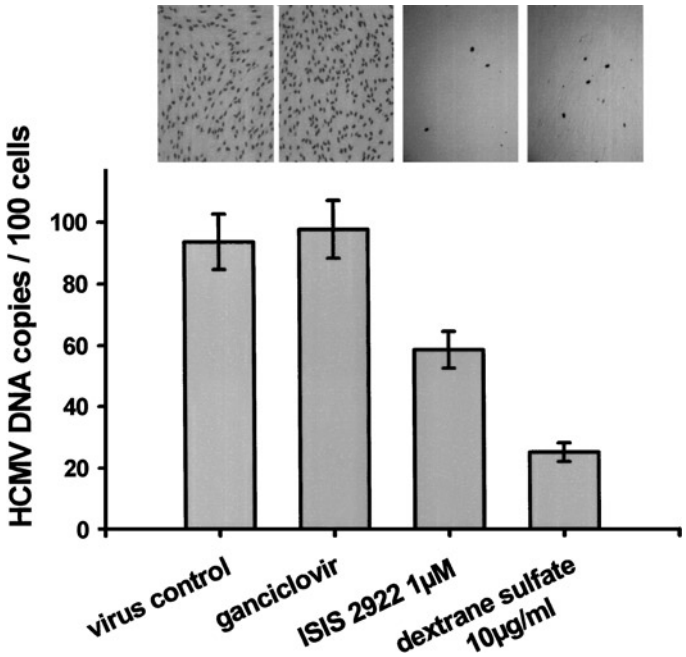

Figure 2. Effects of ISIS 2922 on adhesion/penetration of HCMV to HFF cells infected with MOI 1-2. Corresponding photographs showing IE stained control.

ISIS 2922 prevented both IE/LA antigen expression and HCMV-induced TSP-1 and -2 down-modulation. GCV and ISIS 26062 had no effect on IE and TSP-1 and -2 expression. In all cell types expression of TSP-1 and TSP-2 mRNA was significantly decreased already 24 hrs pi in HCMV-infected compared to mock-infected (results not shown). Using ISIS 2922 at a concentration of 1 μM, it inhibited more than 99% of IEA expression. ISIS 2922 partially inhibited virus adhesion and penetration by 40% in HFF cells (97.5 ± 9.2 DNA copies/100 cells vs. 58.5 ± 5.6 DNA copies/100 cells; $p < 0.05$) compared to virus control. These findings suggest that a significant part of anti-HCMV effects occurred after virus penetration/adhesion. There were no such unspecific effects using GCV, whereas dextrane sulfate showed only inhibition of virus binding/penetration . Because in vivo HCMV spreads from cell to cell, unspecific inhibition of virus adhesion may be less useful than inhibition of virus gene expression. ISIS 2922, but not GCV, is capable to inhibit IEA in a specific mode of action and therefore prevents IE-derived deregulation of cellular genes, which is partially responsible for pathogenic effects.

Although nonspecific effects of ISIS 2922, as suggested (8), cannot be completely excluded, inhibition of both IE and changes in cellular gene expression demonstrate that inhibition of HCMV IE expression by antisense oligonucleotides is a promising strategy to develop novel therapeutic approaches.

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