REGULATION OF THE EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) AND THE PUTATIVE ADHESION MOLECULE BASIGIN ON MURINE CEREBRAL ENDOTHELIAL CELLS BY MHV-4 (JHM)

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INTRODUCTION

Cerebral vascular endothelial cells are integral components of the blood-brain barrier and are believed to be an important site which can restrict infection of the central nervous system by viruses and other pathogens. A potential role of cerebral vascular endothelial cells in resisting CNS infection relates to the expression of lymphocyte-endothelial adhesion molecules which could impact on the generation of anti-viral immune reactivity.

We report here on the regulation of expression of two adhesion related molecules, ICAM-1 and Basigin, following MHV-4 infection. ICAM-1 is a cell surface glycoprotein of the immunoglobulin superfamily expressed on endothelial cells and is involved in interactions with lymphocytes and neutrophils (1, 2). Basigin is the mouse homologue of the chicken antigen HT7, which is expressed on endothelial cells of the blood-brain barrier (3). This antigen is also a member of the immunoglobulin superfamily like ICAM-1 and may play a role in cell adhesion.

METHODS AND RESULTS

Virus Infection of Endothelial Cell Cultures

Cerebral endothelial cell lines were generated from BALB/c (MHV-susceptible) and SJL (MHV-resistant) mice as previously described (4). Endothelial cells grown in T-25 flasks (1×10^6 cells/flask) were treated with MHV-4 or UV-MHV-4 for 1 hour at 37^0C using a multiplicity of infection (MOI) of 0.1. At selected times after infection the endothelial cell cultures were processed for flow cytometry and Northern Analysis to detect ICAM-1 and Basigin.

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Flow Cytometry to Detect ICAM-1

Endothelial cells from infected or paired uninfected cultures were labeled with monoclonal rat anti-mouse ICAM-1 (YN/1.7, obtained from Dr. Fumio Takei, Terry Fox Cancer Center, University of British Columbia, Vancouver, Canada). Fluorescein conjugated goat anti-rat IgG (Organon Teknika-Cappel) was used as a secondary reagent. The percentage of positive cells and mean fluorescence intensities were determined by analysis on the flow cytometer (EPICS C, Coulter Diagnostics, Hialeah, FL, USA) equipped with an argon laser tuned to 488nm. Both MHV-4 and UV-inactivated MHV-4 exposure resulted in a 60% decrease in ICAM-1 expressing endothelial cells (Fig.1)

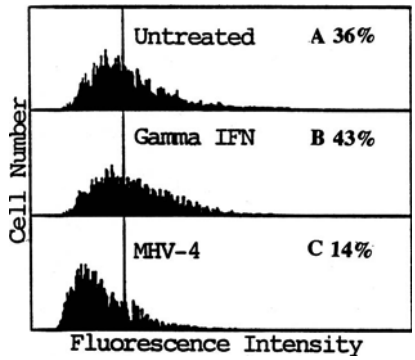

Fig. 1 Fluorescence histograms of ICAM-1 expression on BALB/c cerebral endothelial cells. Flow cytometry profile; X-axis: fluorescence intensity, Y-axis: cell number. Gate windows for green fluorescence lay between channels 0 and 255. (x-axis). Gate windows for red fluorescence was set for exclusion of non viable cells by ethidium bromide. A: Untreated, B: γ-IFN treatment for 72 hours. C: MHV-4 (JHM) treatment for 72 hours.

Northern Analysis to Detect Basigin mRNA Following Virus Infection of Endothelial Cells

BALB/c and SJL derived cerebral endothelial cells were exposed to MHV-4 or UV-inactivated MHV-4 as described above for 24 hours. After these treatments total cellular RNA was isolated from the endothelial cells by guanidinium isothiocyanate/cesium chloride method. RNA was electrophoresed in denaturing formaldehyde gels and blotted by capillary transfer to a zeta probe membrane (Biorad, San Francisco, CA) and hybridized with ^{32}P labeled Basigin cDNA probe (67°C in 7% SDS, 0.5M sodium phosphate, pH 7.2). The blots were washed under high stringency conditions and analyzed by autoradiography. Fig. 2 shows a Northern blot of the results obtained with the Basigin cDNA probe.

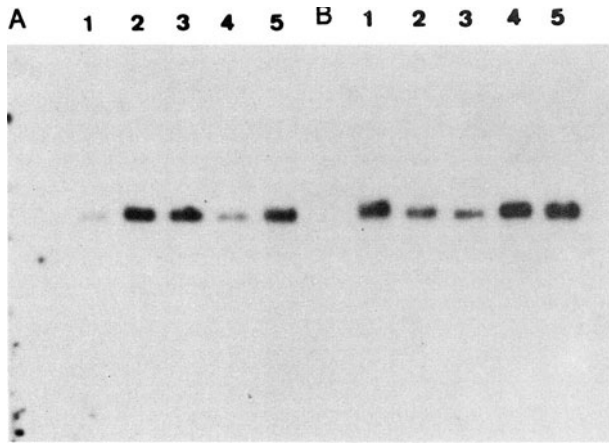

Fig. 2 Northern Blot Analysis of Basigin mRNA induction. Panel A: SJL cerebral microvascular endothelial cells. Panel B: BALB/c cerebral microvascular endothelial cells. Lane 1: Untreated, Lane 2: MHV-4, Lane 3: UV-MHV-4, Lane 4: Tumor necrosis factor, Lane 5: γ-Interferon.

The exposure of brain endothelial cells to MHV-4 or UV-inactivated MHV-4 resulted in dramatic changes in the expression of Basigin mRNA. In MHV-resistant SJL derived brain endothelial cells there was a 240% and 270% increase in Basigin mRNA 24 hours after treatment with MHV-4 and UV-inactivated MHV-4 respectively, as determined by Northern Analysis. In MHV-susceptible BALB/c derived brain endothelial cells, there was a 37% and 26% decrease in Basigin mRNA 24 hours after treatment with MHV-4 and UV-inactivated MHV-4 respectively (These percent changes were calculated by from densitometry analysis of Northern blot, Fig.2).

The mechanism of regulation of brain endothelial cell adhesion molecules by MHV-4 and the impact of changes in their expression on anti-viral immune reactivity is an area of further study.

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