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INDUCTION OF MHC CLASS I ANTIGEN EXPRESSION FOLLOWING INFECTION OF A HUMAN ESTHESIONEUROBLASTOMA CELL LINE WITH CYTOMEGALOVIRUS AND HUMAN IMMUNODEFICIENCY VIRUS

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Summary. – Productive infections with cytomegalovirus (CMV) and human immunodeficiency virus (HIV) were established in the Tp41ON cell line derived from a human esthesioneuroblastoma. HIV antigen expression was highest in cultures coinfected with CMV and HIV. Viral infection caused increased MHC class I antigen expression while class II and CD4 antigens remained undetectable using immunofluorescence methods. Uninfected cultures showed 10 % and coinfected cultures 80 % class I antigen positive cells. In coinfected cultures, CMV and HIV antigens were detected in 4 % and 8 % of the cells, respectively. The detection of CMV antigens in some multinucleated cells suggests coinfection with both viruses in these cells, as multinucleated cells were not found in cultures infected with CMV only. The study shows that a cell line showing neuronal differentiation *in vitro* can be infected with CMV and HIV and that this infection increases MHC class I antigen expression.

Key words: human immunodeficiency virus; cytomegalovirus; MHC class I antigen; esthesioneuroblastoma cell line

Introduction

Central nervous system (CNS) infection with HIV is associated with acute meningitis, subacute meningoencephalitis, myelopathy or the AIDS-related dementia complex (Levy *et al.*, 1985; Navia *et al.*, 1986; Price *et al.*, 1988). While HIV is strongly linked to these states, the viral entry mechanism into the CNS and the type of infected cells are at present unclear. The cellular mechanism which lie behind the CNS disturbances observed are not understood. Normal and neoplastic CNS cells can be infected with HIV *in vitro* but the infection is usually transient and does not cause a cytopathic effect (Chiodi *et al.*, 1987; Dewhurst *et al.*, 1987*a*; Dewhurst *et al.*, 1987*b*). CMV is a major cause of prenatal

neurological damage (Stagno *et al.*, 1982; Johnson, 1982) and is also implicated in some cases of the postinfectious myelo-radiculopathy as well as in encephalites in immunosuppressed patients (Johnson, 1982). HIV and CMV infected brain cells, some of which showed evidence of infection with both viruses, were a constant finding in AIDS patients (Nelson *et al.*, 1988). *In vivo*, CMV has been isolated from epithelial (Wright, 1973), lymphoid and brain tissues (Weller, 1981). *In vitro*, CMV can infect and express early antigens (EA) in a large number of different cell types (Michelson-Fiske *et al.*, 1975; Einhorn and Öst, 1984). Productive CMV replication has, however, only been reported in human cultured fibroblast (Smith, 1956; Smith, 1986), in an embryonal carcinoma cell line (Gönczöl *et al.*, 1984) and in human kidney masangial cells (Heieren *et al.*, 1988).

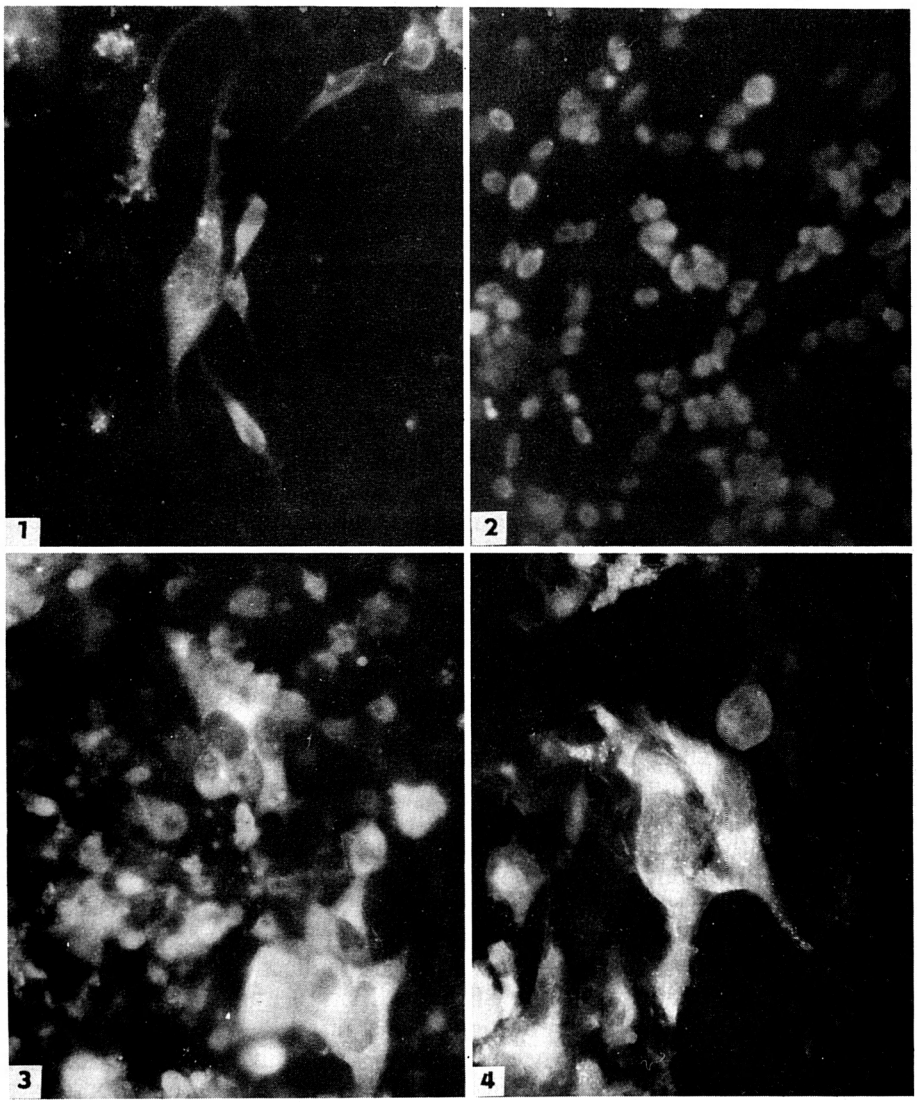
In the present study we show that a cell line with neuronal differentiation can be infected with HIV and CMV and that the infection alters the cellular surface antigen expression.

Materials and Methods

The cell line Tp41ON was established from a surgically removed esthesioneuroblastoma. The line shows immunochemical evidence of expression of neurofilaments (M_r 200 K) and neuron-specific enolase. Sodium channels, characteristic of neurons, were demonstrated in the cellular membranes (Collins, V. P., personal communication). The cells were grown as monolayers on coverslips in sterile 24-well plates (Nunc) with F-10 medium supplemented with 10 % foetal calf serum (FCS; Gibco) or individual human HIV-antibody negative or positive sera (all sera were HIV-antigen negative). The monolayers were infected with 100 $TCID_{50}$ of CMV strain Towne and/or 100 $TCID_{50}$ of HTLV-IIIB for 12 hr and then thoroughly washed. The cultures were evaluated daily for 12 days. The HIV antigen content of the supernatants was measured using a sandwich ELISA which detect the p24 antigen. Aliquots of the supernatants were transferred to human lung cells (HL) for detection of CMV infectivity. The coverslips were fixed in acetone: methanol (1:1) at -20 °C for 5 min, air dried and examined by indirect immunofluorescence technique. The primary antibodies used included anti-CMV EA antibodies (C3631 and rabbit anti-DNA polymerase, supplied by V.-A. Sundqvist, National Bacteriological Laboratory, Stockholm), anti-HIV human serum, anti-CMV human serum (from the NBL), anti-CD4 murine monoclonal antibodies (MoAbs), anti-HLA class I MoAbs (Dako) and anti-HLA DR MoAbs (Becton and Dickinson), respectively. The second antibodies were either fluorescein isothiocyanate (FITC) - conjugated anti-mouse Ig, FITC-conjugated anti-rabbit Ig or FITC-conjugated anti-human Ig antibodies (Dako). A minimum of 1000 cells were assessed in each experiment.

Results

Cytopathic effect was seen in all infected cultures. The HIV infected cultures showed both cytopathic changes, and in some instances, development of syncytia. The controls remained unchanged. The HIV antigen content of the supernatants was measured to quantify viral replication. The volume of tissue culture medium and number of cells were equal in all cultures. The mean concentrations of HIV antigen after 12 days in three cultures are given. The supernatants of HIV and HIV/CMV cell cultures grown in the presence of FCS contained equal amounts (mean 340 ng/ml) of HIV p24 antigen. In the presence



Figs. 1–4

Fig. 1. Tp41ON cells in a CMV/HIV coinfected culture showing immunofluorescence for HLA-ABC antigen.

Fig. 2. Positively stained nuclei of a CMV infected Tp41ON cultures. MoAbs against CMV EA were used.

Fig. 3. Multinucleated cells in a CMV/HIV coinfected culture. Anti-CMV antiserum was used.

Fig. 4. Positive immunofluorescence of Tp41ON cells in an HIV infected culture. Anti-HIV antiserum was used.

of HIV-negative serum, the p24 antigen levels were 490 ng/ml in HIV infected and 640 ng/ml in coinfected cell cultures. In the presence of HIV-positive human serum p24 levels were 630 ng/ml and 760 ng/ml, respectively. Transfer of 500 μl of the supernatant from the CMV infected cultures produced a marked cytopathic effect in the CMV permissive HL cells within 48 hr. Cells stained with the CMV- and HIV-antibody preparations by immunfluorescence are shown in Figs. 2, 3 and 4. In cultures infected with HIV alone, 4 % of the cells showed evidence of HIV antigens by use of immunofluorescence. In the coinfected cultures, that figure rose to 8 %. In cultures infected with CMV alone, 25 % of the cells expressed detectable antigens, the figure dropping to 4 % on coinfection. HLA-ABC expression was seen in 10 % of uninfected cells, increasing to 25 % in CMV or HIV monoinfected and to 80 % in coinfected cultures (Fig. 1). Neither the HLA-DR antigen nor the CD4 antigen were detected by immunofluorescence. The HLA-DR and CD4 expression did not differ between cells grown in the different media with supplements. Both positive and negative controls were included for each step of the assays and gave appropriate results.

Discussion

The Tp41ON cells, derived from a human esthesioneuroblastoma and displaying characteristics of neuronal differentiation could be infected with both CMV and HIV. Previously, HIV infections of both glial and neuronal cells *in vitro* have been reported for a number of cells lines (Chiodi *et al.*, 1987; Dewhurst *et al.*, 1987*a*). Attempts to obtain stable, productive infections have failed. The present study was not aimed at obtaining such a productive infection but rather to test the hypothesis that cells with neuronal differentiation can be infected with HIV. The corroboration of this hypothesis supports the thesis that HIV may reach the CNS not only through mononuclear cell crossing the blood-brain barrier (BBB) but also via neurons as has been shown for both rabies (Johnson, 1982) and herpes viruses (Kristensson *et al.*, 1971; Kristensson *et al.* 1974). In the case of HIV, neuronal infection could primarily occur in any tissue. Even if a lytic infection is not induced, a limited replication in neurons may be sufficient to spread virus inside the BBB. Addition of human serum increased HIV antigen production either by a trophic effect on the cells or through a direct effect on virus replication as has been suggested by Robinson *et al.* (1988). They described two factors increasing HIV replication *in vitro*. One factor was an immunoglobulin present only in HIV seropositive patients and the other was a heat labile factor present in normal human sera. HIV entry into cells not expressing CD4 has been reported (Christofinis *et al.*, 1987) but it is also quite possible that the cells expressed CD4 molecules at levels undetectable by immunofluorescence but still functional. The inability to detect the membrane bound CD4 protein by immunofluorescence has been reported in material shown to express mRNA for this product (Klatzman *et al.*, 1984). A CMV

permissive cell line with neuronal differentiation has not been reported previously. The findings are not unexpected since the CNS may be severely damaged by CMV infection. Nelson *et al.* (1988) found CMV sequences and gene products in glial cells and neurons in HIV infected individuals. The increase of HIV antigen production through CMV/HIV coinfection was hardly surprising, since other herpes viruses have been shown to transactivate HIV (Nelson *et al.*, 1988). We have not double stained the infected cells with anti-CMV and anti-HIV antisera but the demonstration of anti-CMV immunofluorescence in multinucleated cells (Fig. 3) suggests a coinfection with CMV and HIV. Only HIV infected cells were found to become multinucleated *in vitro*. It has been previously reported that HIV, but not CMV, causes multinucleated giant cells *in vitro*.

The dramatic increase of HLA antigen expression may have wide pathogenic implications. In the CNS, cells express very low or no HLA antigens *in vivo* (Lampson and Hickey, 1986; Sobel and Ames, 1988). The Tp41ON cells showed very low levels of these antigens *in vitro*. CMV and/or HIV infection induced HLA expression in up to 80 % of the cells, although only a minority of them expressed viral antigens. The most probable explanation is that soluble factors released into the medium mediated induction of the HLA antigens. Should the present findings apply to the *in vivo* situation, the HLA antigen expression would make the cells susceptible to a class I restricted attack by cytotoxic T cells.

Even an abortive neuronal infection may thus have a tremendous effect upon the function of brain cells, provided that the present *in vitro* data parallel *in vivo* phenomena. A primary infection on the CNS could be established by circumventing the BBB and a secondary effect can be mediated via induction of HLA antigens on infected and nearby neurons, making these cells susceptible for immune attack. However, monocytes and lymphocytes pass through the BBB in an immunological surveilance of the CNS (Leibowitz and Hughes, 1983); the BBB should thus probably not be viewed as an obstacle to CNS spread of viruses showing tropism for these cells.

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