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Involvement of gD/HVEM interaction in NF-kB-dependent inhibition of apoptosis by HSV-1 gD

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ABSTRACT

In the present paper, we aimed to verify whether the interaction of the glycoprotein D (gD) of herpes simplex 1 (HSV-1) with the HSV-1 receptor HVEM is involved in NF-κB-dependent protection against apoptosis by gD. To this purpose, first we utilized MAbs that interfere with gD/HVEM interaction and U937 cells that naturally express human HVEM on their surface. Pre-incubation with these MAbs, but not with a control antibody, partially reverted the protection of infectious HSV-1 towards anti-Fas induced apoptosis in U937 cells. Similarly, pre-incubation of UV-inactivated HSV-1 (UV-HSV-1) or recombinant gD with the same MAbs, significantly reduced the inhibition of Fas-mediated apoptosis by UV-HSV-1 or gD, respectively, in U937 cells. Moreover, coculture with stable transfectants expressing at surface level wild type gD protected U937 cells against Fas-induced apoptosis, while coculture with transfectants expressing a mutated form of gD, incapable to bind HVEM, did not protect. Finally, UV-HSV-1 protected against staurosporine-induced apoptosis in U937 cells as well as in the CHO transfectants expressing human HVEM on their surface, but not in the control CHO transfectants, which did not express HVEM. These results suggest that signaling triggered by binding of gD to HVEM could represent an additional mechanism of evasion from premature apoptotic death exerted by HSV-1-gD in HVEM-expressing cells, disclosing new opportunities of cell death manipulation by using gD preparations.

1. Introduction

Replication and spread of viruses can be efficiently limited by a cellular apoptotic response, that represents an important, first line form of antiviral innate defense (reviewed in [1–3]).[1] Nevertheless, different viruses have evolved multiple survival strategies to escape elimination by apoptosis and interference with signals involved in the death of infected cells is one of the

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more powerful mechanisms for viruses to control non-specific resistance of the host. In fact, regulation of apoptotic pathways is exploited by a wide variety of viruses, either to permit the maintenance of viral infection or to maintain the viability of the host cell, thus enhancing the efficiency of viral replication (reviewed in [4–6]). Particularly, herpes simplex virus 1 (HSV-1) seems able to exert a fine control on apoptosis of infected cells, mainly consisting in prevention of premature cell death to guarantee that a prolonged virus replication could occur (reviewed in [7,8]). In fact, a number of HSV-1 gene products have been proved to play a role in preventing apoptosis. These include the γ134.5 protein, the infected cell protein no. 4 (ICP4), the infected cell protein no. 27 (ICP27), the Us3 protein kinase and, as recently shown, the Us11 protein [9–14]. Moreover, also the envelope glycoprotein D (gD) of HSV-1 has been shown to exert an anti-apoptotic effect [15–17]. Regarding to the latter, we have previously demonstrated that gD of HSV-1, as well as non-replicating UV-inactivated HSV-1, was able to protect U937 cells against Fas-induced apoptosis and that this effect was dependent on activation of NF-kB [17]. However, molecular events involved in cellular signalling upstream NF-kB activation have not been identified.

Binding of the envelope gD with surface cell receptors plays a central role for HSV-1 entry into the cell and for initiating the replication cycle of the virus [18,19]. Receptors for HSV-1-gD include a modified form of heparan sulfate, nectin-1 and nectin-2 and the herpes virus entry mediator (HVEM), a member of the tumor necrosis factor receptor family [19].

We have recently observed that the interaction of gD with HVEM can be involved in NF-kB activation by non-replicating HSV-1 [20]. Thus, we asked whether the same interaction is involved in protection against apoptosis exerted by HSV-1 or its gD in HVEM-expressing cells. To answer this question we tested for a possible role of HVEM in gD-dependent anti-apoptotic activity of HSV-1. Here, we show that, in HVEM-expressing U937 cells, prevention of gD/HVEM interaction by appropriate monoclonal antibodies or by usage of transfectants expressing a mutated form of gD, incapable to bind HVEM, significantly reduced protection against Fas-mediated apoptosis by HSV-1/gD. Moreover, human HVEM expression by stable transfection rendered CHO cells sensitive to HSV-1-induced protection against staurosporine induced apoptosis, whilst control CHO transfectants, which did not express human HVEM, were resistant to such a protective action. Therefore, our results support a role for HVEM in the anti-apoptotic activity of HSV-1/gD.

2. Materials and methods

2.1. Virus and cells

A "F" strain of HSV-1, originally obtained from ATCC, was used in all experiments. Virus stocks were produced, titered in Vero cells and stored in aliquots at −80 °C. To obtain UV-inactivated virus (UV-HSV-1), the HSV-1 suspension was placed in Petri dishes and exposed for 210 s to UV light at an intensity of 30 W using a germicidal lamp situated 10 cm above the sample. Lack of infectivity was tested by titering aliquots of UV-HSV-1 in Vero cells. For experiments of protection against apoptosis by infectious or UV-inactivated virus, approximately 6×10^4 U937 cells in 96-well plates were exposed to virus inoculum for 1 h, at a multiplicity of infection (MOI) of 50 PFU/cell for infectious HSV-1 and, usually, at a MOI of 200 PFU/cell for UV-HSV-1, respectively, 24 h after cultures had been split. The MOI used were chosen on the basis of our previous study [17] and preliminary experiments showing that any further increase in the MOI of UV-HSV-1 failed to improve protection from apoptosis. Subsequently, virus inoculum was replaced by fresh growth medium and cells were subjected to successive experimental procedures.

Vero (originally obtained from the American Type Culture Collection, ATCC) and I143tk$^-$ cells (kindly provided by Prof. Gabriella Campadelli-Fiume, University of Bologna, Bologna) were propagated in Dulbecco's modified Eagle's medium supplemented with 10% FCS (Sigma–Aldrich, St. Louis, MI). CHO cells (originally obtained from the European Collection of Cell Cultures, ECACC) were propagated in HAM'S medium supplemented with 10% of FCS (Sigma–Aldrich). U937 cells (originally obtained from the Istituto Zooprofilattico, Brescia, Italy), were propagated in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 10% FCS (Sigma–Aldrich). Cell lines were cultured at 37 °C in a 5% CO_2 incubator. Cell viability was assessed by a standard trypan blue exclusion test.

2.2. Antibodies and reagents

Anti-gD monoclonal antibody (MAb) 1D3 and purified recombinant gD [gD-1(306t)], were kindly provided by Dr. Gary H. Cohen and Dr. Roselyn J. Eisenberg, University of Pennsylvania, Philadelphia, PA. Anti-gD 1103/H170 MAb, anti-gD HD1 MAb and anti-gC 1104/R633 MAb, were a gift from Prof. Bernard Roizman, University of Chicago, Chicago, IL. MAbs 1D3 and HD1 neutralize HSV infection of cultured cells and blocks HSV-1 binding to HVEM. Anti-HVEM ANC3B7 (SC-65284) MAb was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG was purchased from ICN Biomedicals (Aurora, OH). Anti-human Fas antibody, clone CH11, was purchased from Upstate Biotechnology (Lake Placid, NY). Mouse IgG1 isotype control MAb was purchased from R&D System (Minneapolis, MN). Staurosporine was purchased from Sigma–Aldrich.

2.3. Generation of HVEM expression-construct

An HVEM expression-construct was generated using cDNA obtained from HVEM expressing U937 cells. To this purpose, total RNA was extracted from U937 cells using the "TRIZOL RNA extraction kit" (Gibco). An amount of 1.5 μg of total-RNA was then reverse-transcribed to cDNA using 60 units of AMV reverse transcriptase. The reverse transcription was primed with a mixture of oligo(dT)15 and random hexamer primers and performed in presence of a pool of nucleotides consisting of 1 mM dGTP, dATP, dTTP and dCTP (Promega, Minneapolis, MN). Twenty units of SUPERase-IN (Ambion, Austin, TX) were added to each reaction mixture. The mixture, containing the RNA template and the primers, was first heated at 70 °C for 10 min, chilled on ice, and after the addition of the other components, incubated at 42 °C for 45 min, shifted at 52 °C for 45 min, and