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Modulation of HLA-G and HLA-E Expression in Human Neuronal Cells After Rabies Virus or Herpes Virus Simplex Type 1 Infections

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ABSTRACT: Human Leukocyte Antigen (HLA)-G and E are nonclassical human MHC class I molecules. They may promote tolerance leading to virus and tumor immune escape. We recently described that the herpes simplex virus type 1 (HSV-1), a neurotropic virus inducing chronic infection and neuron latency, and rabies virus (RABV), a neuronotropic virus triggering acute neuron infection, up-regulate HLA-G expression in human neurons (NT2-N). Surface expression was only detected after RABV infection. We investigated here whether RABV and HSV-1 up-regulate HLA-E expression in human neuronal precursors (Ntera-2D/1). We found that RABV, not HSV-1, up-regulates HLA-E expression, nevertheless HLA-E could not be detected on the surface of RABV-infected Ntera-2D/1. Altogether these data suggest that HLA-G and not HLA-E could contribute to the immune escape of RABV. In contrast, there was no evidence that these molecules are used by latent HSV-1 infection. Thus, neurotropic viruses that escape the host immune response totally (RABV) or partially (HSV-1) regulate HLA-G expression on human neuronal cells differentially. *Human Immunology 68, 294–302 (2007).* © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: HLA-G; HLA-E; rabies virus; HSV-1; neurons; Ntera-2-D/1

ABBREVIATIONS

RABV	rabies virus
HSV-1	herpes simplex virus type 1
mAb	monoclonal antibody
HCMV	Human cytomegalovirus

INTRODUCTION

Viruses have developed strategies to facilitate their own dissemination by escaping attack by T and NK cells [1]. Some viruses induce the apoptosis of T or NK cells by increasing the production of immunosubversive molecules in the tissues they infect, resulting in the inactivation of T cells expressing receptors for these molecules. One such molecule, the Ligand of Fas, FasL, has been shown to be involved in the killing of migratory T cells into the nervous system (NS) in rabies virus (RABV) infection [2, 3]. HLA-G, a nonclassical major histocompatibility complex (MHC) molecule, whose expression in cells is up-regulated following infection with human cytomegalovirus (HCMV), human immunodeficiency virus (HIV-1), and RABV could also contribute by a similar mechanism to the immunoevasion of these viruses [4–8]. In addition, HIV-1 and HCMV up-regulate the expression of another nonclassical MHC class I molecule, HLA-E, at the surface of the infected cells leading to the protection of the infected cells from NK lysis [9–13].

Thus, the capacity of HLA-G to kill T and NK cells through the binding to the inhibitory receptor KIR2DL4 expressed by NK cells [14–16] or through the

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binding to CD8 molecules present on T cells [17], and the capacity of HLA-E to modulate NK function via its binding to NKCD94/NKG2A receptors [18–20], has been hijacked by viruses to sneak through the innate and adaptative host immune responses.

HLA-G and HLA-E are two nonclassical Class I (Ib) molecules with a low level of polymorphism. Seven HLA-G isoforms—four membrane-bound (HLA-G1, G2, G3, and G4) and three soluble (HLA-G5, G6, and G7) isoforms—obtained by means of alternative splicing of a single primary transcript [21] have been identified. The most studied are the membrane-bound HLA-G1 and soluble HLA-G5 isoforms. Both are associated with β2 microglobulin [22]. HLA-G, which was originally thought to be present exclusively in the human placenta [23, 24] has also been detected in brain and neural cells [25, 26] including human neurons [8]. HLA-E is ubiquitously transcribed in most human tissues. It is associated with the β2-microglobulin. Surface expression of HLA-E is usually weak suggesting that post translational mechanisms regulate its expression.

The question of whether the HLA-E can be employed by neurons during infection of the NS by viruses that are known to escape the adverse host immune response, such as RABV [2, 3, 8, 27], or not, such as latent strain of HSV-1 (KOS virus), was challenged here. We therefore investigated whether RABV and HSV-1 infected human neurons express HLA-E and whether HLA- E molecules reach the cell surface of the infected neurons.

MATERIALS AND METHODS

Antibodies and Reagents

Mouse monoclonal antibody (mAb) MEM-G/09 specific for the native β2-microglobulin-associated HLA-G forms (corresponding to the native HLA-G1 and HLA-G5 isoforms) [28, 29], MEM-E/07 specific for the native HLA-E, and MEM-E/02 [28] specific for the HLA-E denaturized heavy chain (43 kDa) molecules were from Exbio. Isotype-matched irrelevant mAbs were obtained from Serotec. Biotinylated W6-32 was from Leinco-Biotechnologies. Phycoerythrin (PE)-conjugated streptavidin was purchased from Dako. FITC-conjugated rabbit anti-RABV nucleocapsid Ab was obtained from Biorad. Pan-ERK antibody was from BD Transduction Signal. Fluoromount-G was obtained from Southern Biotechnology Associates. Alexa Fluor 594-conjugated goat anti-mouse Ab was purchased from Molecular Probes. Biotinylated anti-mouse IgG was obtained from Amersham. Cell-fix and Fc-Block (rat anti-Fcγ III/II receptor mAb) were purchased from BD Biosciences. Phosphate Safe extraction Buffer was from Novagen. Protease inhibitor cocktail and Pefabloc SC were obtained from Roche. Hot Start *Taq* polymerase and RNeasy Protect kits were purchased from Qiagen. Superscript II RT was obtained from Invitrogen. Agilent RNA Nano LabChips were purchased from Agilent Technologies. Protran BA83 Cellulose nitrate 0.2μm membranes were from Shleicher-Schuell.

Human Cells and Viruses

Ntera-2D/1 cells (ATCC CRL, 1973) are human neuronal precursors from which human neurons, NT2-N, or mixed cultures of neurons and astrocytes, NT2-N/A, can be differentiated using two different types of differentiation protocols as previously described [8]. M8-pcDNA a HLA-A, B, C, and E positive but HLA-G negative melanoma cell line [30] transfected with a hygromycin resistant vector was used as an HLA-E positive control cell.

The laboratory strain CVS (ATCC vr959), a highly pathogenic RABV strain [31] was propagated as previously described [32]. The latent HSV-1 strain KOS [33] was propagated on U373MG. Cells were infected at a multiplicity of infection (MOI) of three if required.

Analysis of Gene Expression after RABV or HSV-1 Infection

NT2-N/A cells were non-infected or infected with RABV. RNAs were isolated 24 hours later from both infected and non-infected cultures by using the RNeasy Kit. Duplicate samples obtained two weeks apart were used to exclude experimental variations. Control quality was monitored on Agilent RNA Nano Labchips. Gene expression profiles were analyzed using Affymetrix microarrays (U133 plus2.0) containing probe sets representative of the whole human genome. Experiments were done at the Génopole Strasbourg-Alsace-Lorraine (http://www-genopole.u-strasbg.fr/) by Affymetrix standard protocols (http://www.affymetrix.com/support). Detected probe sets were selected according to the "presence calls" and the fold changes were established by Affymetrix software (Microarray Suite v5.0 and Data Mining Tool v2.0). Only significantly changed probe sets ($p < 0.05$) were considered. Up-regulated genes were distributed into clusters of cell functions using Net Affyx and Gene Ontology Mining tool Softwares.

Standard RT-PCR

Total RNA was extracted with RNeasy kits 18 (HSV-1) or 24 hours (RABV) after infection. RNA quality was monitored using Agilent RNA Nano Labchips. The first-strand cDNA was synthesized with oligod (T) primers. 18S RNA was used as a reference (housekeeping gene). Infection efficiency was assessed by PCR, amplifying HSV-1 UL54 or RABV N. RT-PCR was carried out as described in the 13th HLA Workshop report [34] using oligo (dT) primers, and for HLA-E: the primer couples E. 251F: 5′-ACACGGAGCGCCAGGGACAC -E.1272R: