Herpes Simplex Virus Blocks Intracellular Transport of HLA-G in Placentally Derived Human Cells[1]

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Spontaneous fetal loss is associated with herpes simplex virus (HSV) infection as deduced from epidemiologic data. To date, the underlying mechanisms remain to be elucidated, but an immune component is suspected. HLA-G is a class I MHC molecule selectively expressed on extravillous cytotrophoblast; this cell type does not express conventional HLA-A or -B, whereas expression of novel HLA-C-like products has been reported. While its function remains unclear, a role for HLA-G in silencing NK cells that would otherwise attack cells devoid of classical class I molecules has been invoked. We here show that expression of HLA class I molecules is abrogated in HSV-infected choriocarcinoma cells, a phenomenon mediated by the virally encoded inhibitor of the transporter associated with Ag presentation, ICP47. These observations may provide a link between HSV infection and spontaneous fetal loss. ***The Journal of Immunology,*** **1996, 157: 3375–3380.**

The epidemiologic association between herpes simplex virus (HSV)[3] infection and spontaneous pregnancy loss has been well documented (1–4), yet the mechanisms underlying this association remain unclear. A direct viral cytopathic effect is possible, particularly in cases of primary HSV infection during early pregnancy, yet the association remains even in the absence of documented pregnancy-associated primary infection (1–3). Other factors often invoked in fetal loss, such as dysregulation of the maternal immune response, may be equally important. With the recent description of the inhibitory effects of HSV on expression of MHC class I products and on MHC class I-restricted Ag presentation (5, 5a, 6), it is enticing to hypothesize an immune mechanism for HSV-related spontaneous pregnancy loss. More specifically, HSV-related down-regulation of placenta-specific MHC molecules might provoke fetal rejection.

Mammalian placentation provides for the exchange of nutrients between the mother and the fetus. As such, it represents the interface between two genetically disparate individuals. One of the unsolved riddles of immunology is a thriving fetus in an immunocompetent, and usually semiallogeneic, host. Why isn't the fetus rejected in the same manner as an allograft? For a process as essential as reproduction, the solution to the immunologic paradox of pregnancy may involve multiple, overlapping mechanisms, two of which are frequently mentioned. First, the maternal immune system might be modified such that, for the duration of pregnancy, allogeneic responses would be altered, but not suppressed completely (7). The pregnancy-related amelioration of the symptoms of some autoimmune diseases may illustrate such immunomodulation. Second, embryonic cells that contact the maternal circulation could escape maternal immune detection via down-regulation of expression of the appropriate MHC products involved in Ag presentation (8, 9). It is this latter mechanism that we address here.

Recognition of target cells by T cells is guided by products of the MHC. T cells expressing the $\alpha\beta$-type TCR almost invariably use MHC class I or class II products as restricting elements (10, 11). For nonprofessional APCs in humans, the classical HLA-A and -B alleles are used predominately, an observation that may simply relate to their relative abundance at the cell surface. The lack of class I MHC expression would render a cell incapable of presenting Ag to T cells. At the same time, failure to express class I molecules predisposes a cell to NK cell-mediated lysis (12). Thus, during pregnancy, it would be inadequate for invading placental cells (trophoblast) to avoid destruction by maternal alloreactive T cells by simply ablating expression of class I molecules, as this would presumably render these cells a target for lysis by NK cells.

Appropriately, extravillous trophoblast lacks the expression of HLA-A and-B, but expresses the nonclassical class I molecule, HLA-G (13–16), as well as an additional class I molecule thought to be similar, if not identical, to C-locus products (17–19). For the purposes of this report and until incontrovertibly identified, we will hence call this incompletely characterized HLA-C-like polypeptide HLA_{JEG}. The striking sequence similarity of HLA-G to HLA-A,B,C molecules (20) as well as the ability of HLA-G to bind peptides (21) suggest that it may function in a manner analogous to its classical counterparts. An attractive hypothesis would invoke a role for both HLA-G and HLA_{JEG} in mediating protection against NK cells, particularly NK-like large granular lymphocytes present in high numbers in the maternal decidua (22). The simultaneous switching off of the classical class I molecules by trophoblast cells would eliminate inadvertent attack by alloreactive maternal T cells. If correct, inappropriate expression of classical class I molecules or the switching off of HLA-G/HLA_{JEG} would be equally disastrous for fetal survival.

Seen from this perspective, the relationship between spontaneous abortion and the role played by pathogens therein is of considerable interest. HSV and human CMV have been implicated in spontaneous fetal loss (23, 24). Interestingly, these viruses share

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[3] Abbreviations used in this paper: HSV, herpes simplex virus; TAP, transporter associated with antigen presentation; ER, endoplasmic reticulum; MOI, multiplicity of infection; Staph A, *Staphylococcus aureus*; EndoH, endoglycosidase H.

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the unusual ability of interfering with the expression of HLA-A,B molecules, albeit by completely distinct mechanisms (25). This property has evolved presumably to elude destruction of the productively infected cell by cytolytic T cells.

HSV-1 and HSV-2 have been shown to block intracellular transport of classical class I molecules in infected fibroblasts, a function attributable to the HSV immediate early protein, ICP47 (5a, 6). This inhibition of intracellular transport is caused by the failure to provide class I molecules with their essential third subunit, the antigenic peptide (5). ICP47 interdicts the provision of peptide to class I molecules by binding to the transporter associated with Ag presentation (TAP), thus inhibiting transport of peptide across the endoplasmic reticulum (ER). By withholding peptide, stable assembly of MHC class I molecules is blocked. The relevance of HSV's immunoevasive activity to HLA-G and trophoblast immune function has not been previously addressed. We here show that in an HLA-G-expressing tumor cell line of extra-embryonic origin, the choriocarcinoma cell line JEG-3, infection with HSV inhibits peptide transport and down-regulates HLA-G and HLA_{JEG} expression, a function that can be attributed to the action of ICP47. These observations provide a possible link between fetal loss and HSV infection.

Materials and Methods

Cell lines

JEG-3 choriocarcinoma cells, HeLa cervical carcinoma cells, and CCD18 Lu human lung fibroblasts (all from American Type Culture Collection, Rockville, MD) were maintained in DMEM supplemented with 10% FCS, 2 mM L-glutamine, 1/1000 dilution units/ml penicillin, and 100 μg/ml streptomycin. All cells were cultured in a 5% CO_2 environment at 37°C, and all cell culture materials were obtained from Life Technologies (Gaithersburg, MD).

Antibodies

The mAb W6/32 recognizes conformationally correct products of the HLA-A, -B, -C (26, 27), and -G (28) loci complexed to β_2m. The mAb 66Ig10 (29) recognizes the human transferrin receptor. The rabbit antiserum anti-ICP47–1 (6) was raised against a carboxyl-terminal peptide of ICP47. The rabbit antiserum anti-TAP1 (30) was raised against a carboxyl-terminal domain of TAP 1.

Viruses and viral infection

Recombinant vaccinia virus expressing both TAP-1 and TAP-2 (VV-TAP 1&2) was a generous gift from J. Bennink and J. Yewdell, National Institutes of Health (Bethesda, MD). Construction of VV-TAP 1&2 has been previously described (5). Cells were infected with VV-TAP 1&2 at a multiplicity of infection (MOI) of 5 for 1 h in a small volume of PBS supplemented with 10% BSA (Boehringer Mannheim, Indianapolis, IN) at 37°C. DMEM supplemented with 10% FCS, glutamine, penicillin, and streptomycin was then added to cultures for an additional 2.5 to 3 h at 37°C before metabolic labeling and analysis.

The recombinant adenovirus expressing ICP47 (AdICP47–1) and the adenovirus with deletion of the E1 region (Add1E1) were generous gifts from D. Johnson, McMaster University (Ontario, Canada). Generation of AdICP47–1 and Add1E1 has been described in detail (6). Cells were infected with adenoviral strains at an MOI of 50 in a small volume of PBS/10% BSA at 37°C. DMEM supplemented with 10% FCS, glutamine, penicillin, and streptomycin was then added to cultures for an additional 36 h at 37°C before metabolic labeling and analysis.

Mutants of HSV-2, strain G, and HSV-1, strain 17, with deletions of the UL41 host protein shutoff gene (31) were the kind gifts of R. Everett and D. McGeoch, Medical Research Council Institute of Virology (Glasgow, U.K.). These mutant viruses were used to optimize metabolic labeling of HLA-G. Cells were infected with the HSV mutants at a MOI of 5 to 10 in a small volume of PBS/10% BSA for 1 h at 37°C. Infection medium was then supplemented with DMEM/10% FCS/L-glutamine/penicillin/streptomycin for an additional 4 h before metabolic labeling and analysis.

Biochemical analysis and gel electrophoresis

Cells were metabolically labeled with [^{35}S]methionine in cysteine/methionine-free DMEM (Life Technologies). The amounts of label and duration of labeling are described in the figure legends. Comparable numbers of control cells were always treated in parallel. Metabolically labeled cells were lysed in Nonidet P-40 lysis mix (0.5% Nonidet P-40, 50 mM Tris-HCl, and 5 mM $MgCl_2$, pH 7.3) containing 1 mM PMSF (32) on ice for 30 min. Total cell lysates were cleared of debris by centrifugation, and supernatants were precleared three times with 3 μl/ml normal rabbit serum and 3 μl/ml normal mouse serum. Immune complexes were removed after each preclearing by absorption with 100 μl of 10% fixed *Staphylococcus aureus* (Staph A). Precleared lysates were immunoprecipitated with specific antisera as described, and immune complexes were collected on Staph A or protein A (Sigma Chemical Co., St. Louis, MO). Immunoprecipitated products were washed four times with ice-cold wash buffer (0.5% Nonidet P-40; 50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 5 mM EDTA; and 0.1% SDS). Samples were then resuspended in sample buffer (62.5 mM Tris-HCl, pH 6.8; 5% 2-ME; 10% glycerol; 4% SDS; and bromophenol blue) and boiled at 95°C for 5 min before SDS-PAGE (33). In those experiments in which endoglycosidase H (EndoH; New England Biolabs, Beverly, MA) was used, washed Staph A- or protein A-bound immune complexes were processed according to the manufacturer's instructions. Briefly, immune complexes were denatured in 0.5% SDS, 1% 2-ME at 95°C for 10 min, then exposed to EndoH in 50 mM sodium citrate (pH 5.5) at 37°C for 60 min. Samples were mixed with 4× sample buffer and boiled for 3 min before SDS-PAGE.

Pulse-chase analysis

Approximately 6×10^6 JEG-3 choriocarcinoma cells were infected with VV-TAP 1&2 for 2.5 h as described, then starved in cysteine/methionine-free DMEM for 30 min at 37°C. Cells were metabolically labeled with 2.0 mCi [^{35}S]methionine for 30 min at 37°C and washed in DMEM/10% FCS. Cells (1.5×10^6) were then removed and lysed in Nonidet P-40 lysis buffer as the pulse/0 min chase aliquot. The remainder of labeled cells continued in culture at 37°C until removal of equivalent numbers of cells for 30, 60, and 120 min chase points. Cell lysates were processed for immunoprecipitation as described.

Transport assays

Transport assays were performed as previously described (5). Briefly, JEG-3 cells were trypsinized and washed three times in DMEM/10% FCS and once in transport buffer (130 mM KCl, 10 mM NaCl, 1.0 mM $CaCl_2$, 2.0 mM EGTA, 2.0 mM $MgCl_2$, and 5.0 mM HEPES, pH 7.3, with KOH) at 4°C. Cells (10^6/assay) were then permeabilized by resuspension in 80 μl of transport buffer containing 2 U/ml streptolysin O (Bio-Merieux, Marcy l'Etoile, France). Cells were incubated at 37°C for 10 min to obtain >80% permeabilization, as assessed by loss of trypan blue exclusion. A 10-μl aliquot of an ^{125}I-labeled peptide library was added to all assay tubes before 0- or 10-min incubation at 37°C. For inhibition of TAP-specific transport, synthetic ICP47 (B. Galocha, A. B. Hill, and H. L. Ploegh, manuscript in preparation) was added to a concentration of 10 μM. Samples of synthetic ICP47 are available on request. Peptide transport was stopped by the addition of 1.0 ml of ice-cold stop buffer (transport buffer plus 10 mM EDTA). Cells were collected by centrifugation and lysed as described previously. Glycosylated peptides were collected from the lysis supernatant by adsorption to 100 μl of Con A-Sepharose (Pharmacia, Uppsala, Sweden) during a 1-h incubation at 4°C. Con A-Sepharose beads were washed four times in lysis buffer, and radioactivity was measured by gamma spectrometry.

Results

Detection of HLA-G and TAP in choriocarcinoma cells

The choriocarcinoma cell line JEG-3 expresses HLA-G and little, if any, classical HLA-A and -B products (34). Labeling of JEG-3 cells with [^{35}S]methionine and immunoprecipitation with the W6/32 Ab (Fig. 1*B*) readily reveals HLA_{JEG}, the 44-kDa class I heavy chain observed previously in this cell line and presumed to be HLA-C-like (17–19), and the 39-kDa membrane-bound form of HLA-G (21, 35). Likewise, constitutive expression of TAP-1 and TAP-2 in JEG-3 cells was documented (Fig. 1*A*) in immunoprecipitation experiments using anti-TAP Ab. These data complement previous reports (36, 37) and demonstrate levels of TAP expression in JEG-3 cells to be intermediate between those in human lung fibroblasts and those in HeLa cervical carcinoma cells (based on equal amounts of labeled starting material).

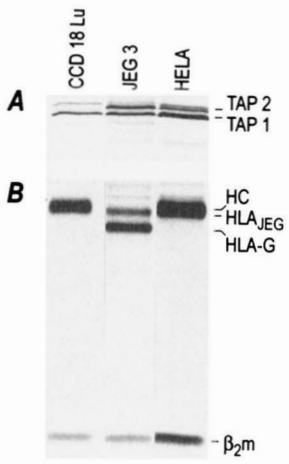

FIGURE 1. MHC class I products and TAP components are expressed constitutively in JEG-3 choriocarcinoma cells. *A*, The levels of TAP-1 and TAP-2 in JEG-3 choriocarcinoma cells are intermediate between those in human lung fibroblasts (CCD18 Lu) and those in cervical carcinoma cells (HeLa) as demonstrated through metabolic labeling of approximately 10^7 cells with 500 μCi ^{35}S overnight and subsequent immunoprecipitation with polyclonal anti-TAP-1 Ab. *B*, HLA products are expressed as a doublet in immunoprecipitations with W6/32, with a presumed HLA-C-like product (HLA$_{JEG}$) at 44 kDa and the membrane-bound form of HLA-G at 39 kDa. Classical class I MHC heavy chains (HC) are demonstrated for comparison at approximately 46 kDa in CCD18 Lu cells and HeLa cells.

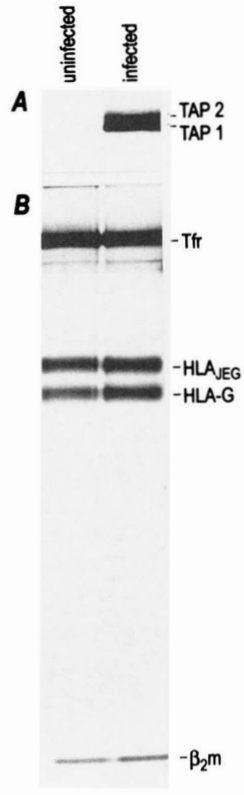

FIGURE 2. TAP-1 and TAP-2 expression in JEG-3 cells is enhanced by infection with VV-TAP-1&2 without changes in W6/32-reactive MHC class I products or other constitutive cellular proteins. *A*, JEG-3 choriocarcinoma cells were infected with VV-TAP-1&2 as described, metabolically labeled with 500 μCi ^{35}S for 2 h, and immunoprecipitated with anti-TAP-1 Ab. Both TAP-1 and TAP-2 are dramatically overexpressed, as shown even at exposure times for which constitutive levels of TAP proteins in uninfected cells are difficult to detect. *B*, Overexpression of TAP-1 and TAP-2 does not appreciably affect either levels of transferrin receptor synthesis (Tfr) as detected by immunoprecipitation with 66Ig10- or W6/32-reactive HLA-G or HLA$_{JEG}$.

Level of TAP is not a rate-limiting step in maturation of HLA-G molecules

It has been argued that the supply of peptide may be the rate-limiting step in the control of assembly of class I molecules (38). Sources of peptide other than those delivered by TAP have been considered and include the utilization of signal sequence-derived peptides as well as peptides delivered to the lumen of the ER by transporters other than TAP (39, 40). Furthermore, cellular factors that could regulate the activity of TAP might play a role in determining overall rates of peptide transport. To date, the relative levels of TAP and the activity of TAP have not been assessed quantitatively for any human cell lines. In our experiments, immunoprecipitation from control JEG-3 cells revealed a low, but detectable, level of the TAP complex (Fig. 1*A*), the activity of which was noted in peptide transport assays (Fig. 4). We used recombinant vaccinia virus vectors to drive the expression of TAP-1 and TAP-2 subunits to high levels. If the activity of TAP is a rate-limiting step in the assembly and transport of HLA-G or HLA$_{JEG}$, we should observe an increase in the relative amounts of W6/32-reactive class I molecules as well as an accelerated rate of acquisition of EndoH resistance. The acquisition of complex N-linked sugars during the maturation of glycoproteins occurs as the protein traverses the medial Golgi and results in resistance to digestion with EndoH. Thus, the presence of EndoH sensitivity indicates that protein maturation has not proceeded past the ER. While infection with VV-TAP-1&2 resulted in at least a 10-fold increase in the amount of labeled TAP subunits precipitated (Fig. 2*A*), the amount of labeled transferrin receptor, the amount of W6/32-reactive HLA-G/HLA$_{JEG}$ (Fig. 2*B*), and the relative amounts of EndoH-resistant material in pulse-chase analysis (Fig. 3) were comparable for VV-TAP-1&2-infected and noninfected cells. Overexpression of TAP-1 and TAP-2 affected neither HLA-G nor HLA$_{JEG}$ in terms of reactivity with W6/32 or rate of acquisition of EndoH resistance. We conclude that in JEG-3 cells, the level and activity of the TAP complex are such that a further increase in the levels of TAP does not measurably affect the assembly or rate of transport of class I molecules.

TAP complex is active and can be inhibited by ICP47 in choriocarcinoma cells

To confirm that the TAP complex present in JEG-3 cells is functional, we performed a peptide translocation assay. The activity of TAP and its inhibition by ICP47 can be measured in vitro by making use of streptolysin-permeabilized cells and a radiolabeled translocation substrate. This translocation substrate consists of a peptide library, each component of which contains a glycosylation consensus sequence allowing ER retention of any translocated peptides. Such peptides can then be retrieved using Con A-Sepharose. Peptide translocation in JEG-3 choriocarcinoma cells was readily demonstrable (Fig. 4), as has been shown for other adherently grown cells (5). Since it has been suggested that the effects of ICP47 might show some degree of cell type specificity, with fibroblasts being more susceptible to such inhibition than lymphoid cells (6) (A. B. Hill and H. L. Ploegh, unpublished observations), it was important to establish that the activity of TAP in JEG-3 cells is susceptible to inhibition by ICP47. We have prepared a synthetic and fully biologically active version of ICP47, an 87-residue peptide (B. Galocha, A. B. Hill, and H. L. Ploegh, manuscript in preparation). Addition of synthetic ICP47 to the in

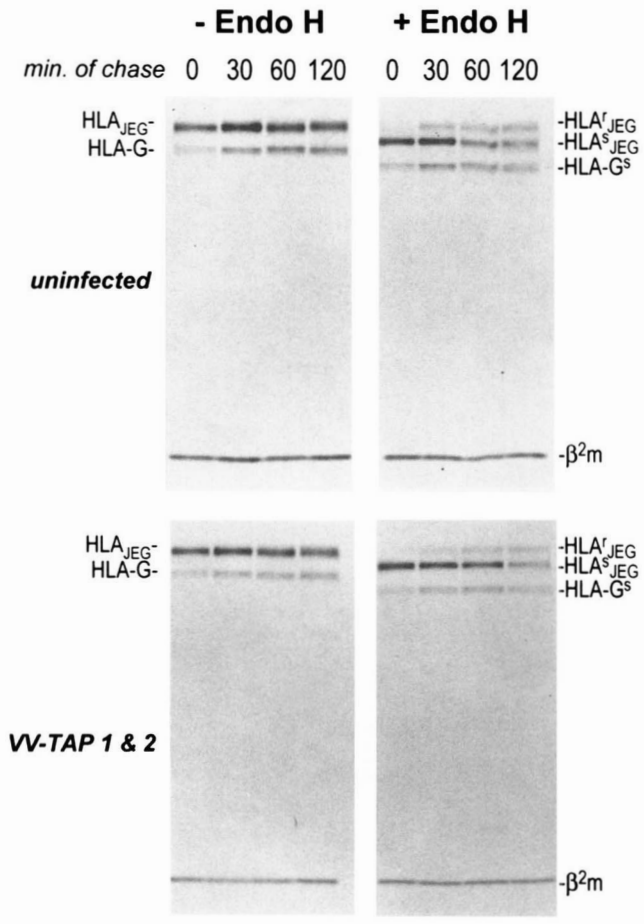

FIGURE 3. Infection of JEG-3 choriocarcinoma cells with VV-TAP-1&2 does not affect HLA-G maturation as measured by EndoH sensitivity in pulse-chase analysis. JEG-3 choriocarcinoma cells infected with VV-TAP-1&2 for 3 h, pulsed with ^{35}S for 30 min, and chased with unlabeled medium for the times indicated. In the absence of EndoH treatment, both HLA-G and HLA$_{JEG}$ accumulate over the 120-min chase period with identical kinetics in infected and uninfected cells. In the presence of EndoH, the EndoH-resistant form of HLA$_{JEG}$ (HLA$_{JEG}^r$) accumulates over the chase period with similar kinetics in infected and uninfected cells. The EndoH-sensitive forms of HLA$_{JEG}$ (HLA-G$_{JEG}^s$) and HLA-G (HLA-G^s) increase for the first 60 min of chase, then decrease. Again, kinetics are similar in uninfected and infected cells. VV-TAP-1&2 infection causes a decrease in total cellular protein synthesis; therefore, film exposure times for infected cells were increased to allow approximately comparable signal strengths for figure presentation.

vitro translocation assay abolishes transport in JEG-3 semi-intact cell preparations. The activity of TAP in choriocarcinoma cells is, therefore, susceptible to inhibition by ICP47, as is the case in fibroblasts.

HSV-infected JEG-3 cells retain HLA-G in the ER

The transport of newly synthesized class I MHC through the ER to the Golgi can be assessed by the predictable acquisition of EndoH resistance. Susceptible cells infected with HSV are no longer capable of such transport. This specific inhibition of intracellular trafficking is caused by the HSV-encoded immediate early protein, ICP47, which induces a failure to deliver peptide to the ER via the TAP transporter (5, 6). We observed that W6/32-reactive HLA-G/HLA$_{JEG}$ from JEG-3 cells infected with HSV remained almost completely EndoH sensitive, while that from noninfected cells acquired EndoH resistance (Fig. 5). It is known that the W6/32 Ab,

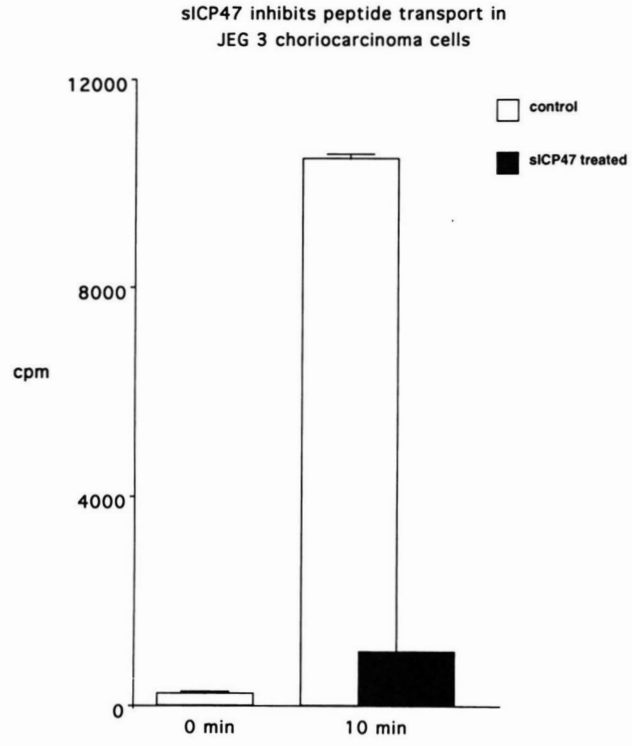

FIGURE 4. Synthetic ICP-47 (sICP47) inhibits peptide transport across the ER as measured by transport assays in JEG-3 choriocarcinoma cells. Minimal background activity is noted for transport of a ^{125}I-labeled peptide substrate across the ER at time zero. After 10 min, appreciable labeled peptide is transported across the ER, retained there via glycosylation, and detected by retrieval on Con A-Sepharose. The addition of 10 μM sICP47 nearly abrogates peptide transport at 10 min.

while specific for properly conformed class I molecules, does react with class I complexes devoid of peptide. The block in class I transport and maturation is seen for both HLA-G and HLA$_{JEG}$, and occurs in JEG-3 cells infected with either HSV-1 or HSV-2 (data for HSV-1 not shown). In contrast, processing of transferrin receptor isolated from the same labeling experiments is identical in both infected and noninfected cells (Fig. 5), indicating that the effects of HSV infection on class I maturation are specific.

Infection of JEG-3 cells with AdICP47 abolishes intracellular transport of HLA-G

By making use of a recombinant adenovirus that drives the expression of ICP47, AdICP47, we examined more directly whether the inhibition of intracellular transport of HLA-G and HLA$_{JEG}$ in the JEG-3 cell line was caused by the TAP inhibitor ICP47. JEG-3 cells infected with AdICP47 express ICP47, as shown by immunoprecipitation of metabolically labeled cells with a polyclonal Ab against ICP47 (Fig. 6*B*). As noted in HSV-infected cells, HLA-G and HLA$_{JEG}$ in AdICP47-infected JEG-3 cells fails to acquire EndoH resistance indicating that its transport from the ER to the medial Golgi is inhibited (Fig. 6*A*).

Discussion

Overexpression of TAP in JEG-3 choriocarcinoma cells, as brought about by infection with recombinant vaccinia virus, does not influence the rate of intracellular transport of either HLA-G or HLA$_{JEG}$. We, therefore, conclude that the endogenous levels of TAP in JEG-3 cells suffice to serve these novel class I products

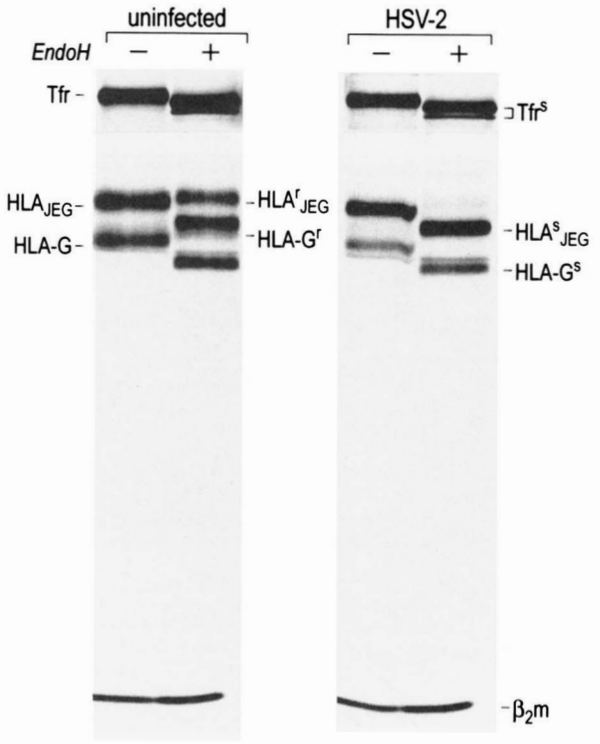

FIGURE 5. Infection of JEG-3 choriocarcinoma cells with HSV results in retention of HLA-G in the ER. While uninfected cells labeled with 500 μCi ^{35}S for 2 h display both EndoH-resistant (HLA-G^r and HLA$_{JEG}$^{r}) and EndoH-sensitive (HLA-G^s and HLA$_{JEG}$^{s}) forms of HLA class I products, HLA-G and HLA$_{JEG}$ retain near complete EndoH sensitivity when JEG-3 cells are infected with herpes simplex virus type 2. HSV-2 infection has no effect on cellular processing of transferrin receptor (Tfr), as EndoH treatment of immunoprecipitated material from both infected and uninfected cells reveals identical EndoH-sensitive forms of Tfr (Tfr^s).

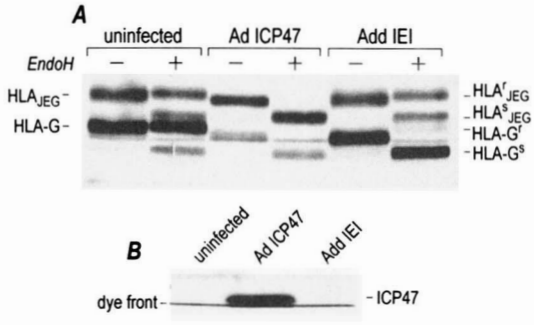

FIGURE 6. Expression of ICP47 by recombinant adenovirus in JEG-3 cells results in the retention of EndoH sensitivity by both HLA-G and HLA$_{JEG}$. *A*, Cells infected with recombinant adenovirus as described were labeled with 500 μCi ^{35}S for 8 h before immunoprecipitation. Uninfected cells display both EndoH-resistant (HLA-G^r and HLA$_{JEG}$^{r}) and EndoH-sensitive (HLA-G^s and HLA$_{JEG}$^{s}) forms of W6/32-reactive class I products, while those infected with recombinant adenovirus expressing ICP47 (AdICP47) only retain EndoH-sensitive forms. Infection with control adenovirus, Add1E1, has little effect on HLA$_{JEG}$, compared with that in uninfected cells, but does shift the processing of HLA-G toward the EndoH-sensitive form. *B*, ICP47 is detected by immunoprecipitation with anti-ICP47 Abs only in those cells infected with AdICP47.

with a pool of peptides adequate for intracellular transport and delivery to the cell surface. Using assays established to assess TAP function, peptide transport in JEG-3 choriocarcinoma cells was readily detected and could be blocked by ICP47, confirming the involvement of TAP in the transport process measured. If the data obtained here for an established choriocarcinoma cell line can be extrapolated to extravillous trophoblast, we draw the following conclusions. First, HLA-G and HLA$_{JEG}$ require a source of TAP-dependent peptides in the same manner as HLA-A and -B products. This substantiates earlier suggestions made on the basis of extensive sequence similarity between HLA-G and classical class I molecules (13). In addition, the ability of HLA-G to bind peptides delivered by TAP was recently demonstrated directly in lymphoblastoid cells transfected with HLA-G (21). Thus, while the physiologic pattern of HLA expression in trophoblast may be unique, its mode of peptide acquisition is identical with that reported for HLA-A and -B molecules.

Second, the possible regulation of the TAP complex by cytosolic or other factors does not appear to be tissue or cell specific. Intracellular transport of HLA products in choriocarcinoma cells is abolished by infection with HSV, and this effect is attributable to the inhibitory effect of ICP47 on the TAP peptide transporter. Both HLA-G and HLA$_{JEG}$ are equally affected in HSV-infected cells. Thus, the inhibitory effects of ICP47 are not confined to fibroblasts, but extend to lymphoid cells (A. B. Hill and H. L. Ploegh, unpublished results) and choriocarcinoma cells. The availability of synthetic ICP47 as an inhibitor that is selective for TAP-dependent peptide transport may find application in other systems where down-regulation of class I molecules has been observed, but where the underlying causes have not been identified. Moreover, the synthesis of any agent that would interfere with the ICP47-TAP interaction should prove beneficial for elimination of virally infected cells. Restoration of peptide transport using such inhibitory agents would render HSV-infected cells visible to appropriate immune effectors. Rescue of MHC class I expression would be expected to occur for all TAP-dependent class I molecules, including HLA-G and HLA$_{JEG}$.

It is important to delineate the implications of the data presented here from a clinical standpoint. We have demonstrated that inhibition of TAP leads to elimination of both HLA-G and HLA$_{JEG}$ from the surface of those cells that normally express them. Because these class I products are expressed at the materno-fetal interface, they would seem ideally positioned to silence NK cells that could attack extravillous trophoblast normally devoid of classical class I products. Such elimination would, therefore, allow the direct attack of fetal tissue by maternal effector cells. In support of this, the transfection of HLA-G into a human B cell line has been shown to confer resistance to decidual NK cell-mediated lysis (22). While we do not yet know the extent of involvement of HLA-G- or HLA-C-like products in the protection of the fetus from immune attack by maternal lymphoid cells, our observations could provide a reasonable explanation for the correlation between HSV infections and spontaneous abortions. If the expression of novel class I products on trophoblast is indeed indispensable for survival of the fetus, then ablation of its expression should not be without consequence. In the presence of HSV infection, the protective effects of these products would no longer manifest themselves, and immune-mediated fetal loss would occur. Other viruses known to be capable of down-regulation of class I molecules in general, and HLA-G in particular, should be examined for possible associations with spontaneous abortions. As shown here, the ability of HSV to down-regulate HLA-G/HLA$_{JEG}$ should be considered established and may provide a long-sought link between spontaneous abortions and infection with HSV.

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