Adenoviral-mediated gene transfer of ICP47 inhibits major histocompatibility complex class I expression on vascular cells in vitro

Louise Furukawa, MD, Lucy S. Brevetti, MD*, Sandra E. Brady, BS, David Johnson, PhD, Madison Ma, BS, Theodore H. Welling, BS, and Louis M. Messina, MD, *San Francisco, Calif*

Purpose: Many viruses have evolved mechanisms to evade detection by the host immune system. The herpes simplex gene ICP47 encodes a protein that binds to the host antigen-processing transporter, inhibiting the formation of major histocompatibility complex class I (MHC-I) antigens in infected cells. MHC-I antigen expression is also important in acute allograft rejection. This study was designed to quantitate the effect of adenoviral-mediated gene transfer of ICP47 on MHC-I cell surface expression of human vascular cells. We hypothesized that the transduction of vascular cells with a replication-incompetent adenoviral vector that was expressing ICP47 (AdICP47) would inhibit constitutive and inducible MHC-I expression and thereby reduce the rate of cytolysis of ICP47-transduced vascular cells by sensitized cytotoxic T lymphocytes (CTL).

Methods: A replication-incompetent adenoviral vector, AdICP47, was created to express ICP47 driven by the cytomegalovirus immediate early promoter. Cultured human vascular endothelial and smooth muscle cells and human dermal fibroblasts were transduced with either AdICP47 or the control empty vector AddlE1. Cell surface constitutive and γ-interferon–induced MHC-I expression were quantitated by flow cytometry. A standard 4-hour chromium release cytotoxicity assay was used to determine the percent cytolysis of transduced and nontransduced endothelial cells by sensitized CTL. Finally, to quantitate the specificity of the effect of ICP47 on MHC-I expression, adhesion molecule expression was quantitated in both transduced and nontransduced cells.

Results: Constitutive MHC-I expression in AdICP47-transduced endothelial cells was inhibited by a mean of 84% ± 5% (SEM) in five experiments. After 48 hours of exposure to γ-interferon, AdICP47-transduced cells exhibited a mean of 66% ± 8% lower MHC-I expression than nontransduced cells. Similar inhibition in MHC-I expression was achieved in AdICP47-transduced vascular smooth muscle cells and dermal fibroblasts. Percent cytolysis of AdICP47-transduced endothelial cells by CTL was reduced by 72%. Finally, the specificity of the effect of transduction of ICP47 on vascular cell MHC-I expression was confirmed by a lack of significant change in either constitutive or tumor necrosis factor–induced vascular cell adhesion molecule / intercellular adhesion molecule expression.

Conclusion: Transduction of vascular cells with AdICP47 strongly inhibits both constitutive and inducible MHC-I expression in human vascular cells. AdICP47-transduced cells exhibited a substantial reduction in cytolysis by CTL. Thus AdICP47 transduction holds promise as a technique to characterize the role of MHC-I expression in acute vascular allograft rejection in vivo and as a potential therapeutic intervention. (J Vasc Surg 2000;31:558-66.)

From the University of California, San Francisco.
Competition of interest: nil.
Supported by The Pacific Vascular Research Foundation, San Francisco, California and National Institutes of Health Grant RO1-HL51184-04.
*The Von Liebig Foundation Research Fellow.
Reprint requests: Dr Louis M. Messina, M488, University of California, San Francisco, 505 Parnassus Ave, San Francisco, CA 94143-0222.

0741-5214 / 00 / $12.00 + 0 24 / 1 / 102591
doi:10.1067 / mva.2000.102591

The major histocompatibility complex class I (MHC-I) protein is a ubiquitous molecule that is expressed on all nucleated cells. MHC-I antigen presentation initiates the detection and clearance of foreign cells of virally infected host cells by $CD8^+$ T lymphocytes. In nature, several viruses have evolved mechanisms to evade host immune detection and destruction by interfering with the normal production or expression of MHC-I antigens.

Under normal circumstances, peptides destined to be present on MHC-I molecules are generated by ubiquitin-dependent proteosomes in the cytosol. A transmembrane protein known as the transporter of antigen-processing protein (TAP) mediates transport of the cytosolic peptides into the lumen of the endoplasmic reticulum. Herpes simplex virus (HSV) expresses an immediate-early protein, ICP47, a 9-kDa protein that binds to the peptide-binding site of the TAP. Once ICP47 is bound to the TAP, translocation of cytosolic peptides into the lumen of the endoplasmic reticulum is inhibited.[1-3] The formation of stable cell-surface MHC-I complexes requires the trimolecular association of class I heavy chain, β_2 microglobulin, and the peptide within the lumen of the endoplasmic reticulum. Without the peptide, the class I heavy chain and the β_2 microglobulin remain misfolded and unstable and thus are unable to be expressed on the cell surface.

With the use of this strategy, the HSV is able to inhibit host cell-surface MHC-I expression in a variety of cells. Within hours of infection by HSV, fibroblasts become resistant to cytolysis by anti-HSV cytotoxic T lymphocytes (CTL).[4,5] When $CD8^+$ T cells recognize foreign MHC-I antigens, an inflammatory cellular infiltration is stimulated. Cytokines such as interleukin-1 are secreted. Also, the expression of thrombomodulin and intercellular adhesion molecule-1 (ICAM-1) is stimulated. Thus downregulation of MHC-I antigens by ICP47 could potentially reduce allograft rejection mediated by $CD8^+$ T cells.

Coronary and peripheral arterial bypass grafts typically are performed with autologous saphenous veins. Synthetic small diameter grafts have inferior patency. Venous allografts have been explored as an alternative when suitable autogenous conduit is unavailable. Unfortunately, the patency rates of both fresh and cryopreserved allografts have been inferior to those of autologous conduit. Rejection as evidenced by an inflammatory cellular infiltrate has been observed.[6-12] Immunosuppression, such as with cyclosporine, reportedly improved patency.[6,10,12-14]

This study was designed to quantitate the effect that adenoviral-mediated gene transfer ICP47 into human endothelial cells has on cell surface MHC-I expression to explore the potential of the ICP47 gene to modulate vascular allograft rejection. We hypothesized that transduction of vascular cells with a replication-incompetent adenoviral vector that expresses ICP47 (AdICP47) would inhibit constitutive and inducible MHC-I expression and thereby reduce the rate of cytolysis of ICP47-transduced vascular cells by sensitized CTL. Gamma-interferon (γ-IFN) was used to induce MHC-I expression because its mechanism of increasing the transcription rate of class I antigens in cultured human endothelial cells is well documented.[15] These studies are the necessary first step to the establishment of the feasibility of adenoviral-mediated gene transfer of ICP47 with the aim of inhibiting acute vascular allograft rejection.

MATERIAL AND METHODS

Cell lines and medium. Human iliac artery endothelial cells were derived from discarded aortoiliac segments of organ donors by dispase (Boerhinger Mannheim, Indianapolis, Ind) digestion at 40 IU / mL. Verification of endothelial identity was confirmed by morphologic feature (monolayer growth pattern, polygonal shape, growth pattern exhibiting contact inhibition), dil Ac-LDL-uptake (Biomedical Technologies, Stoughton, Mass), and staining with monoclonal antibody to vonWillebrand factor. Endothelial cells were propagated in complete medium composed of M199, 20% heat-inactivated fetal bovine serum (Hy Clone, Logan, Utah), 15 U / mL heparin (Sigma, St Louis, Mo), 25 μg / mL endothelial cell growth supplement (Collaborative Biomedical, Bedford, Mass), 100 U / mL penicillin, 100 μg / mL streptomycin, and 5 mmol / L L-glutamine (Gibco, Germantown, Md). Endothelial cells were grown on 1% gelatin-coated tissue culture–treated polystyrene flasks. Human aortic smooth muscle cells and human dermal fibroblasts were obtained through American Type Culture Collection (Rockville, Md) and propagated in minimum essential medium with 10% heat-inactivated fetal bovine serum, 100 U / mL penicillin, 100 μg / mL streptomycin, and 5 mmol / L L-glutamine.

Viral vectors. A replication-incompetent adenoviral vector containing the ICP47 gene was constructed as described previously.[16] Briefly, the herpes simplex 1 gene ICP47 driven by the human cytomegalovirus (HCMV) immediate-early promoter was cloned into the E1-deleted region of an E1, E3-deleted adenoviral backbone and called AdICP47-1.

(From here forward, the vector described as AdICP47-1 will be called AdICP47.) AdhpAP is similar to AdICP47 except that the expression cassette is a reporter gene that encodes human placental alkaline phosphatase. This gene was selected as a reporter to quantitate transduction efficiency, because the protein is heat stable above 65°C and can be distinguished from endogenous alkaline phosphatase by heat inactivation. AddlE1, the control empty vector, is essentially identical to AdICP47 and AdhpAP, except without the expression cassette. All adenovirus was amplified in the permissive human embryonic kidney 293 line (Microbix, Ontario, Canada). After viral purification with cesium chloride, viral stocks were dialyzed against 3% sucrose and stored at –80°C.

Transductions. Vascular cells were grown in T-25 flasks and were transduced at 80% confluence at a multiplicity of infection (MOI) of 100 plaque-forming units (pfu) / cell in a total volume of 1.5 mL of complete medium. After a 4-hour transduction period, excess virus was aspirated. The cells were rinsed in buffered saline solution and fed either complete medium or complete medium supplemented with 200 IU / mL human recombinant γ-IFN (Gibco). After 48 hours, cell surface MHC-I expression was quantified by flow cytometry.

Flow cytometry and antibodies. All flow cytometry was carried out on a FACSCALIBUR (Becton-Dickinson, San Jose, Calif). Cells were stained in 96-well V-bottom microtiter plates with approximately 1×10^5 cells / well in three replicates. Fluorescein isothiocyanate–conjugated anti–HLA-A, -B, -C antibody, phycoerythrin-conjugated anti–HLA-DR, -DP, -DQ, and appropriate isotypic controls were purchased through PharMingen (San Diego, Calif). Anti-CD3, -CD4, -CD8, and -CD56 were purchased through Becton Dickinson (San Jose, Calif). A random sample of 10,000 events was collected per replicate. Monoclonal antibody against vonWillebrand factor was obtained from Dako (Carpenteria, Calif).

Lymphocyte sensitization. Lymphocytes (responder cells) of haplotype HLA-A 11 and 24 and HLA-B 60 and 62 were sensitized to endothelial cells (stimulator cells) of haplotype HLA-A 1 and 24 and HLA-B 44 and 61 by the following method. Lymphocytes were derived from whole blood by Ficoll gradient with Histopaque 1077 (Sigma). Endothelial cells were grown to confluence on 1% gelatin-coated wells of a 24-well plate (Becton Dickinson). Lymphocytes suspended in Yssel's medium with 1% human AB serum (Gemini, Calabasas, Calif) were added to the endothelial cells at an effector-to-target ratio of 5:1. After 4 days of sensitization, human recombinant interleukin-2 (Sigma) was added at a concentration of 40 U / mL. After 7 days of sensitization, the lymphocytes were harvested and resuspended at a ratio of 4×10^5 cells / mL in Yssel's medium.[17,18] The sensitized lymphocytes require restimulation with feeder cells for normal growth. The feeder cells were comprised of irradiated (4000 rad) human peripheral blood lymphocytes at a concentration of 2×10^6 cells / mL and irradiated (5000 rad) JY cells at a concentration of 2×10^5 cells / mL.[19] Four days after the addition of feeder cells, 40 U / mL of human recombinant interleukin-2 was added. Sensitized lymphocytes were used as effectors in a standard 4-hour chromium release cytotoxicity assay 7 days after the addition of the stimulator cells.

Cytotoxicity assay. Endothelial cells that had been transduced with either the control empty vector (AddlE1) or AdICP47 were harvested from their flasks with trypsin and counted. Each cell type was labeled with 100 μCi sodium chromate per million cells for a 2-hour labeling period. Cells were rinsed twice and recounted. Then 2500 targets were added to each well of a V-bottom 96-well plate to which effector cells were added at effector-to-target cell ratios of 100:1, 50:1, 25:1, or 12.5:1. To obtain minimum counts (from spontaneous release), culture medium was added to target cells. To obtain maximum counts, Triton-X 1% in water was added to target cells. Target and effector cells were incubated at 37°C for 4 hours, after which time the microtiter plate was spun at 1000 rpm, and 100 μL supernatant was removed and added to 100 μL scintillation cocktail (Wallac, Gaithersburg, Md).

Immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out on a 15% gel. After transfer onto a 0.2 μm nitrocellulose membrane and overnight blocking with a 5% dry milk solution in Tris buffered saline solution-Tween, primary antibody against ICP47 was added and followed by a biotin-linked goat anti-rabbit secondary antibody (Jackson Immunolabs, West Grove, Pa). Color was developed with an Enhanced Chemiluminescence (Amersham, England) and autoradiography.

Adhesion molecule expression. Human iliac artery endothelial cells were grown on 1% gelatin-coated T-25 flasks to quantitate the effect of ICP47 on adhesion molecule expression. At 80% confluence, the cells were transduced with either the con-

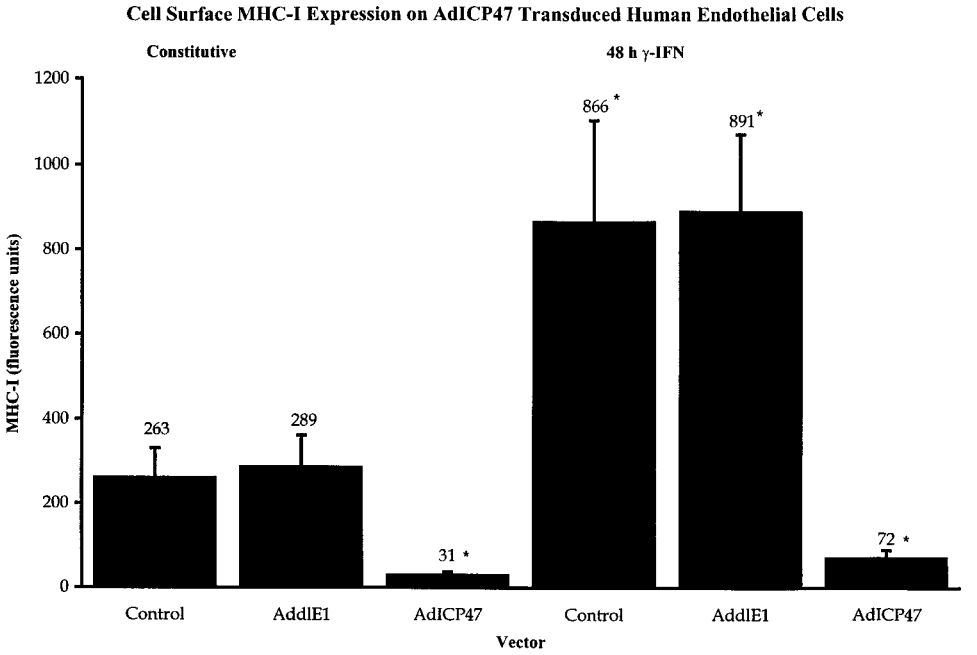

Fig 1. Transduction of human endothelial cells with AdICP47 resulted in a mean inhibition of constitutive MHC-I by 84% (range, 74%-96%). On induction with γ-IFN, nontransduced and AddlE1-transduced cells increased MHC-I expression by 250% and 229% of constitutive expression, respectively. AdICP47-transduced cells exhibited a mean reduction in MHC-I expression 66% lower than did constitutive nontransduced endothelial cells. Data are expressed as the mean ± SEM of five experiments that were performed in triplicate. *$P < .05$ compared with constitutive nontransduced endothelial cells by the Wilcoxon signed rank test.

trol empty vector, AddlE1, or AdICP47 at MOI of 100 pfu / cell. After a 4-hour transduction period, excess virus was aspirated. The cells were rinsed in buffered saline solution and fed either complete medium or complete medium supplement with 100 U / mL human recombinant tumor necrosis factor-α (TNF-α; Sigma). After 48 hours, ICAM-1 and vascular cell adhesion molecule-1 (VCAM) expression was quantified by flow cytometry. Cells were stained in 96-well V-bottom microtiter plates with approximately 1×10^5 cells / well in three replicates. Fluorescein isothiocyanate-conjugated (mouse IgG1, K) and phycoerythrin-conjugated (mouse IgG1) were purchased through PharMingen. A random sample of 10,000 events was collected per replicate.

Statistical analysis. The Friedman's Test was used, comparing the mean percent change in cell fluorescence and the percent cytotoxicity to assess the significance between the multiple groups. The Wilcoxon Signed Rank Test was used to find the significant differences.

RESULTS

Transduction efficiency. To determine whether adenoviral vectors could infect and transduce human endothelial cells, we used AdhpAP. Infection of human endothelial cells with the reporter transgene AdhpAP led to a nearly 100% transduction rate determined by histochemical staining (results not shown).

Inhibition of cell-surface MHC-I on AdICP47-transduced human endothelial cells. In five experiments, the transduction of human endothelial cells with an AdICP47 vector at an MOI of 100 pfu / cell resulted in a mean inhibition of constitutive MHC-I expression by 84% ± 4.8% (mean ± SEM), with a range of 74% to 96% reduction ($P < .05$; Fig 1). No significant difference in MHC-I expression was observed between the nontransduced and AddlE1-transduced endothelial cells. After the cells were exposed to 200 IU / mL of γ-IFN for 48 hours, both the nontransduced and AddlE1-transduced endothelial cells had an increase in MHC-I by 250% and 229% of constitutive, respectively. The

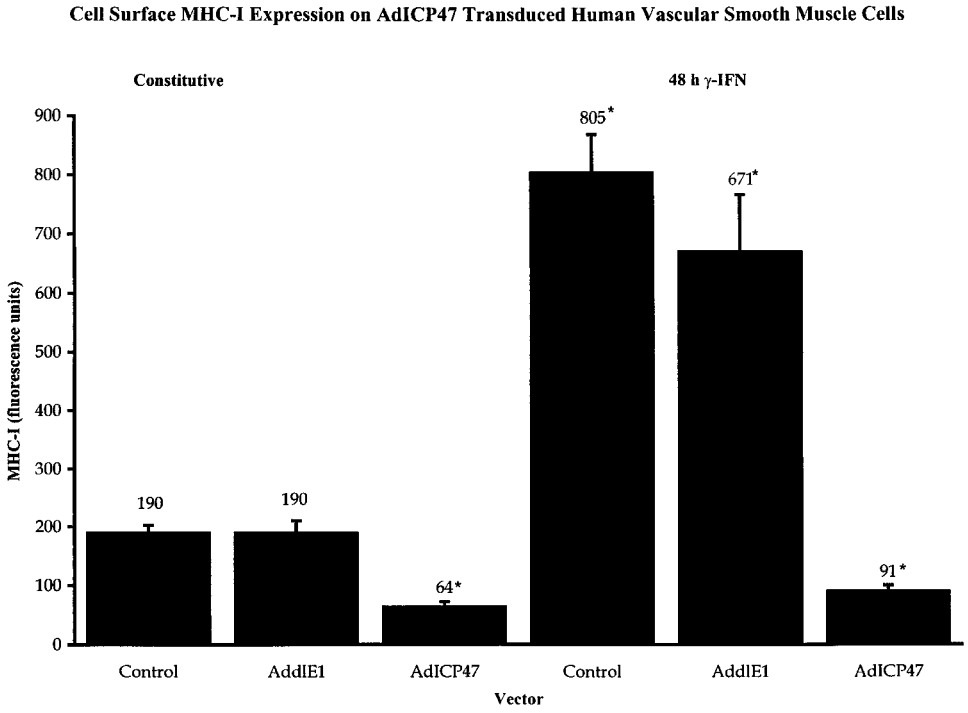

Fig 2. Transduction of human aortic vascular smooth muscle cells with AdICP47 resulted in a mean inhibition of constitutive MHC-I expression by 67% (range, 60%-74%). On induction with γ-IFN, nontransduced and AddlE1-transduced cells increased MHC-I expression by 328% and 267% of constitutive expression, respectively. AdICP47-transduced cells exhibited a mean inhibition in cell-surface MHC-I expression 51% lower than did constitutive nontransduced cells. Data are expressed as the mean ± SEM of five experiments that were each performed in triplicate. $^{*}P < .05$ compared with constitutive nontransduced vascular smooth muscle cells by the Wilcoxon signed rank test.

MHC-I expression of AdICP47-transduced endothelial cells after γ-IFN remained 66% ± 8%, below that of constitutive nontransduced endothelial cells. Thus near complete inhibition of both constitutive and γ-IFN induced MHC-I expression was achieved by transduction with AdICP47.

Inhibition of cell-surface MHC-I expression on AdICP47-transduced human vascular smooth muscle cells. In five experiments, the transduction of human vascular smooth muscle cells with AdICP47 resulted in a 67% ± 2.7% lower MHC-I expression than did the nontransduced cells ($P < .05$). No significant difference existed between the level of MHC-I on nontransduced and AddlE1-transduced smooth muscle cells. After exposure of the cells to γ-IFN for 48 hours, the MHC-I expression on nontransduced and AddlE1-transduced smooth muscle cells increased by 328% and 267% of constitutive, respectively. AdICP47-transduced smooth muscle cells exhibited an 89% ± 0.9% lower MHC-I expression than did the nontransduced smooth muscle cells exposed to γ-IFN (Fig 2). Thus delivery of ICP47 into vascular smooth muscle cells also results in a substantial reduction in both constitutive and γ-IFN–induced MHC-I expression.

Inhibition of cell-surface MHC-I expression on AdICP47-transduced human dermal fibroblasts. In five experiments, the transduction of human dermal fibroblasts with AdICP47 resulted in a mean inhibition of constitutive MHC-I expression by 80% ± 2.9% (range, 70%-87%; $P < .05$). No significant difference existed between the level of MHC-I expression on the nontransduced and AddlE1-transduced fibroblasts. After exposure to 200 IU/mL of γ-IFN for 48 hours, both the nontransduced and AddlE1-transduced dermal fibroblasts increased MHC-I expression by 222% and 185% of constitutive, respectively. AdICP47-transduced dermal fibroblasts exhibited an 88% ± 1.6% lower MHC-I expression than did the nontransduced dermal fibroblasts exposed to γ-IFN (Fig 3).

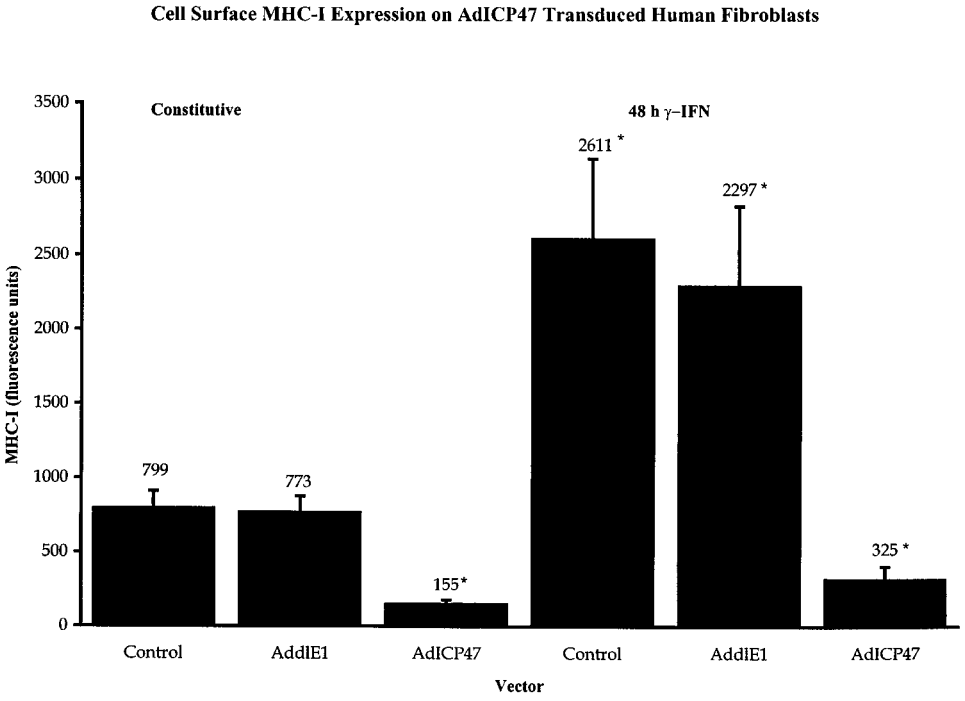

Fig 3. Transduction of human dermal fibroblasts with AdICP47 results in a mean inhibition of constitutive MHC-I expression by 80% (range, 70%-87%). On induction with γ-IFN, nontransduced and AddlE1-transduced dermal fibroblasts increased MHC-I expression by 222% and 185% of constitutive expression, respectively. AdICP47-transduced fibroblasts exhibited a mean inhibition of MHC-I expression 59% lower than did constitutive nontransduced cells. Data are expressed as mean ± SEM of five experiments that were each performed in triplicate. $^{*}P < .05$ compared with constitutive nontransduced fibroblasts by the Wilcoxon signed rank test.

AdICP47-transduced endothelial cells are protected from cytolysis by CTL. With the use of a standard 4-hour chromium release cytotoxicity assay, the cytotoxicity of a polyclonal group of sensitized lymphocytes was tested against nontransduced, AddlE1-transduced, and AdICP47-transduced endothelial cells. At all effector-to-target ratios tested in seven experiments, percent cytolysis of AdICP47-transduced endothelial cells was 46.5% to 71.15% lower than that of either nontransduced or AddlE1-transduced cells ($P < .02$; Fig 4). No significant difference in cytolysis was found between the nontransduced and AddlE1-transduced endothelial cells.

The ICP47 protein is identified in AdICP47-transduced endothelial cells. With the use of standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting, the presence of the ICP47 protein was verified in AdICP47-transduced endothelial cells with appropriate negative bands in the nontransduced and AddlE1-transduced groups (Fig 5).

Effect of AdICP47 transduction on VCAM/ICAM expression. To determine the specificity of the effect of the transduction of ICP47 on vascular cell MHC-I expression, we sought to compare constitutive and TNF-induced VCAM/ICAM expression of transduced and nontransduced cells. There were no differences in constitutive or TNF-induced VCAM expression between AddlE1 or AdICP47 transduced endothelial cells (Table I). The constitutive ICAM expression was also not significantly changed in the AdICP47-transduced endothelial cells from the AddlE1-transduced cells. Finally, the TNF-induced ICAM expression was not significantly different in the AdICP47-transduced groups compared with the nontransduced groups.

DISCUSSION

This study shows that the transduction of human endothelial cells, vascular smooth muscle cells, and dermal fibroblasts with AdICP47 results in potent inhibition of the expression of MHC-I under both constitutive and γ-IFN–induced conditions. Fur-

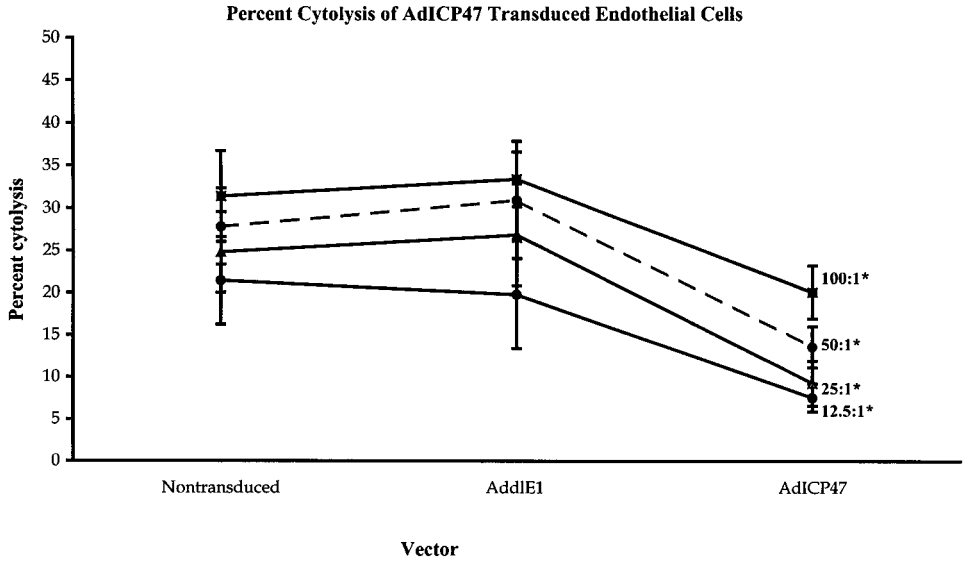

Fig 4. At each effector-to-target cell ratio, percent cytolysis of AdICP47-transduced endothelial cells is between 47% and 71% lower than that of either nontransduced or AddlE1-transduced cells. $^{*}P < .02$. Percent cytolysis between nontransduced and AddlE1-transduced endothelial cells was not significant.

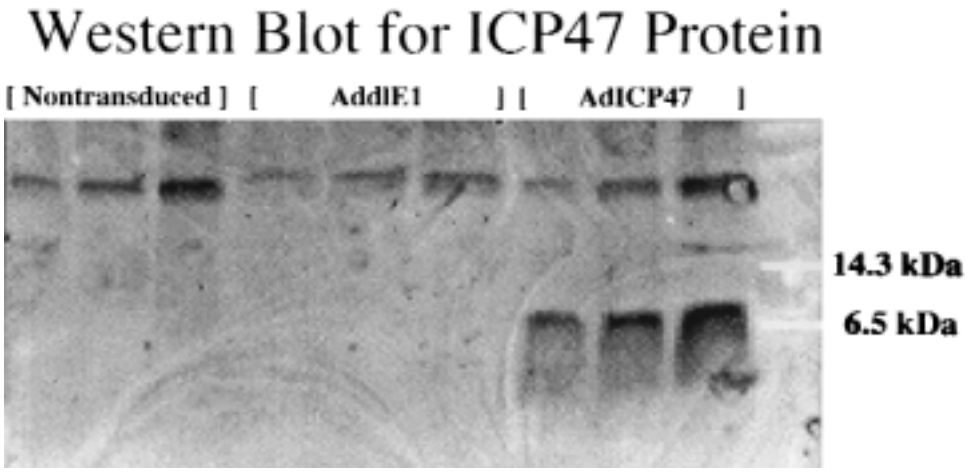

Fig 5. Western blotting reveals positive bands in the 9-kDa range in the lanes corresponding to lysate from AdICP47-transduced endothelial cells. No signal is present in lanes containing lysate from nontransduced or AddlE1-transduced endothelial cells.

thermore, this ICP47-induced reduction in MHC-I expression was biologically significant. The ICP47-transduced cells were protected against cytolysis by sensitized T lymphocytes in a standard cytotoxicity assay. This ICP47-induced inhibition of MHC-I expression appeared to be specific. Although it is well known that adenoviral transduction of endothelial cells causes a cellular activation as seen with VCAM / ICAM,[20] the ICP47 transgene did not significantly change the expression of such mediators.

In light of the findings of this study, several questions must be addressed. First, is the magnitude of reduction in MHC-I sufficient to produce an effect in vivo? Unfortunately, it is not yet known how many MHC-I molecules are needed to initiate a response by CTL. However as few as 500 MHC-II molecules are sufficient to stimulate a proliferative response to allogeneic cells. Therefore the dramatic

reduction in surface class I that we observed, coupled with relatively low endogenous levels of class I molecules in endothelial cells, suggests that ICP47 could reduce $CD8^+$ T-cell responses to vascular allografts. The results of the experiments that involved cytotoxicity of sensitized T cells indicate that recognition by $CD8^+$ T cells is reduced. Still, there is some MHC-I remaining on the cells, probably because of TAP-independent mechanisms. One method to rectify this problem is to express the HCMV gene, US2, in cells. US2 is one of the unique short regions on the HCMV that encodes a glycoprotein that causes the degradation of MHC-I heavy chains.[21] Downregulation of MHC-I may be best achieved by combining the effects of ICP47 and US2.

A second important issue is whether this degree of downregulation of MHC-I leads to the induction of tolerance. This question remains largely unanswered and is related to a third issue of whether this reduction in MHC-I molecules will result in an increased response of natural killer (NK) cells. It is certainly possible that the NK-cell response will become an important means of eliminating allogenic cells that express ICP47. However, not all MHC-I is completely inhibited by the vector. Recently, a second strategy, one of decoy, used by the HCMV to evade host NK cell recognition and destruction has been discovered.[22] The HCMV UL-18 gene encodes a protein product homologous to the MHC-I heavy chain. This molecule is recognized by NK cells and serves as a decoy that inhibits NK cell lysis of the infected cell. If our in vivo system indicates substantial NK cell response, we would choose to use a hybrid vector containing the UL-18, US2, and ICP47.

The downregulation of MHC-I prolonging allograft survival was demonstrated by Efrat et al.[23] In this study, the E3 region of the adenovirus was introduced into pancreatic islet cells of transgenic mice. The E3 region contains the gp 19K gene, which downregulates MHC-I expression by binding specifically to class I antigens as they are being transported to the cell surface.[24] These MHC-I–depleted pancreatic islet cells were transplanted into allogenic mice. Prolonged allograft survival was noted without any other type of additional immunosuppressant. Use of the gp19K gene can, however, be problematic because it binds to different haplotypes with differing affinities. The ICP47 gene product binds to TAP. In some haplotypes, that will not interfere with the level of MHC-I cell-surface expression. However, studies with ICP47 have been slowed because ICP47 does not effectively block the murine TAP[2] so that porcine or primate models are required to test its efficacy in vivo.

Table I. Effect of AdICP47 on endothelial cell VCAM / ICAM expression

	Constitutive mean	*TNF-induction mean*
VCAM		
Nontransduced	3.99	239.00
AddlE1	6.00*	138.59*
AdICP47	5.73*	150.76*
ICAM		
Nontransduced	12.35	2169.67
Add1E1	158.40*	1640.27
AdICP47	173.20*	3073.30†

*$P < .05$ versus nontransduced.
†$P < .05$ versus AddlE1.

We chose to reduce MHC-I on endothelial cells because this strategy has been used effectively in nature by the adenovirus, HSV, and HCMV to evade host immune responses. Our preliminary studies with these three genes US11, gp19K, and ICP47 revealed most pronounced reduction in MHC-I with the ICP47 gene (data not shown). Very few studies have been undertaken to test the efficacy of these gene products on endothelial cells or other components of the vascular wall. An adenoviral vector was chosen to introduce ICP47 into these cells for several reasons. Adenoviruses are known for their high transduction efficiency. The effect of expressed adenoviral proteins and immune destruction of the vector might be abrogated by the immune-suppressant effect of the ICP47 gene. Although adenoviral transduction does not result in integration into the host genome[25] and endothelial cells have a half-life measure in years,[26] it is possible that the induction of tolerance may occur. CTL would fail to recognize and lyse the adenoviral-transduced cell with limited MHC-I expression and thus prolong the lifespan of the adenoviral-infected cell.

In summary, these results show that the transduction of vascular endothelial and smooth muscle cells and dermal fibroblasts with AdICP47 results in a significant specific reduction in both constitutive and γ-IFN–induced MHC-I on the major cell constituents of the vascular wall. Furthermore, AdICP47-transduced endothelial cells exhibited a substantial reduction in cytolysis by CTL, which is specific for MHC-I molecules on the cell surface. The extent of AdICP47-induced MHC-I inhibition and its impact on acute vascular rejection remains to be established in an appropriate in vivo model.

REFERENCES

1. York IA, Roop C, Andrews DW, Riddell SR, Graham FL, Johnson DC. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell 1994;77:525-35.
2. Tomazin R, van Schoot NE, Goldsmith K, Jugovic P, Sempé P, Früh K, et al. Herpes simplex virus type 2 ICP47 inhibits human TAP but not mouse TAP. J Virol 1998;72:2560-3.
3. Ahn K, Meyer TH, Uebel S, Sempé P, Djaballah H, Yang Y, et al. Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus ICP47. EMBO J 1996;15:3247-55.
4. Koelle DM, Tigges MA, Burke RL, Symington FW, Riddell SR, Abbo H, et al. Herpes simplex virus infection of human fibroblasts and keratinocytes inhibits recognition by cloned CD8+ cytotoxic T lymphocytes. J Clin Invest 1993;91:961-8.
5. Posavad CM, Rosenthal KL. Herpes simplex virus-infected human fibroblasts are resistant to and inhibit cytotoxic T-lymphocyte activity. J Virol 1992;66:6264-72.
6. Wagner E, Roy R, Marois Y, Douville Y, Guidoin R. Fresh venous allografts in peripheral arterial reconstruction in dogs: effects of histocompatibility and of short-term immunosuppression with cyclosporine A and mycophenolate mofetil. J Thorac Cardiovasc Surg 1995;110:1732-44.
7. Couvelard A, Leseche G, Scoazec JY, Groussard O. Human allograft vein failure: immunohistochemical arguments supporting the involvement of an immune-mediated mechanism. Hum Pathol 1995;26:1313-20.
8. Nataf P, Guettier C, Martinelli L, Regan M, Pavie A, Cabrol C, et al. Cryopreserved small-diameter arterial allografts for arterial by-pass procedures: an experimental study. J Cardiovasc Surg (Torino) 1995;36:79-85.
9. Wagner E, Guidoin R, Marois M, Mantovani D, Roy R, Ricci M, et al. Histopathologic findings in synthetic and biologic explanted grafts used in peripheral arterial reconstruction. ASAIO J 1994;40:M279-83.
10. Mingoli A, Edwards JD, Feldhaus RJ, Hunter WJ 3rd, Naspetti R, Cavallari N, et al. Fresh vein allograft survival in dogs after cyclosporine treatment. J Surg Res 1996;62:95-102.
11. Augelli NV, Lupinetti FM, el Khatib H, Sanofsky SJ, Rossi NP. Allograft vein patency in a canine model: additive effects of cryopreservation and cyclosporine. Transplantation 1991;52:466-70.
12. Posner MP, Makhoul RG, Altman M, Kimball P, Cohen N, Sobel M, et al. Early results of infrageniculate arterial reconstruction using cryopreserved homograft saphenous conduit (CADVEIN) and combination low-dose systemic immunosuppression. J Am Coll Surg 1996;183:208-16.
13. Miller VM, Bergman RT, Gloviczki P, Brockbank KG. Cryopreserved venous allografts: effects of immunosuppression and antiplatelet therapy on patency and function. J Vasc Surg 1993;18:216-26.
14. da Gama AD, Sarmento C, Vieira T, do Carmo GX. The use of arterial allografts for vascular reconstruction in patients receiving immunosuppression for organ transplantation. J Vasc Surg 1994;20:271-8.
15. Johnson DR, Pober JS. Tumor necrosis factor and immune interferon synergistically increase transcription of HLA class I heavy- and light-chain genes in vascular endothelium. Proc Natl Acad Sci U S A 1990;87:5183-7.
16. Hill A, Jugovic P, York I, Russ G, Bennink J, Yewdell J, et al. Herpes simplex virus turns off the TAP to evade host immunity. Nature 1995;375:411-5.
17. Pardi R, Bender JR. Signal requirements for the generation of CD4+ and CD8+ T-cell responses to human allogeneic microvascular endothelium. Circ Res 1991;69:1269-79.
18. Strong DM, Ahmed AA, Thurman GB, Sell KW. In vitro stimulation of murine spleen cells using a microculture system and a multiple automated sample harvester. J Immunol Methods 1973;2:279-91.
19. Paliard X, Yssel H, Blanchard D, Waitz JA, deVries JE, Spits H. Antigen specific and MHC nonrestricted cytotoxicity of T cell receptor alpha beta+ and gamma delta+ human T cell clones isolated in IL-4. J Immunol 1989;143:452-7.
20. Gerszten RE, Luscinskas FW, Ding HT, Dichek DA, Stoolman LM, Gimbrone MA Jr, et al. Adhesion of memory lymphocytes to vascular cell adhesion molecule-1–transduced human vascular endothelial cells under simulated physiological flow conditions in vitro [see comments]. Circ Res 1996;79:1205-15.
21. Jones TR, Sun L. Human cytomegalovirus US2 destabilizes major histocompatibility complex class I heavy chains. J Virol 1997;71:2970-9.
22. Leong CC, Chapman TL, Bjorkman PJ, Formankova D, Mocarski ES, Phillips JH, et al. Modulation of natural killer cell cytotoxicity in human cytomegalovirus infection: the role of endogenous class I major histocompatibility complex and a viral class I homolog. J Exp Med 1998;187:1681-7.
23. Efrat S, Fejer G, Brownlee M, Horwitz MS. Prolonged survival of pancreatic islet allografts mediated by adenovirus immunoregulatory transgenes. Proc Natl Acad Sci U S A 1995;92:6947-51.
24. Burgert HG, Kvist S. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. Cell 1985;41:987-97.
25. Berkner KL. Expression of heterologous sequences in adenoviral vectors. Curr Top Microbiol Immunol 1992;158:39-66.
26. Schwartz SM, Benditt EP. Cell replication in the aortic endothelium: a new method for study of the problem. Lab Invest 1973;28:699-707.

Submitted May 11, 1999; accepted Aug 6, 1999.