Molecular Medicine 5: 606-616, 1999

Molecular Medicine

Herpes Simplex Virus (HSV)-Mediated ICAM-1 Gene Transfer Abrogates Tumorigenicity and Induces Anti-Tumor Immunity

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Accepted August 20, 1999.

Abstract

Background: Costimulatory and cellular adhesion molecules are thought to be essential components of antigen presentation in the immune response to cancer. The current studies examine gene transfer utilizing herpes viral amplicon vectors (HSV) to direct surface expression of adhesion molecules, and specifically evaluate the potential of a tumor-expressing intercellular adhesion molecule-1 (ICAM-1) to elicit an anti-tumor response.
Materials and Methods: The human ICAM-1 (hICAM1) gene was inserted into an HSV amplicon vector and tested in a transplantable rat hepatocellular carcinoma and in a human colorectal cancer cell line. Cell surface ICAM-1 expression was assessed by flow cytometry. Lymphocyte binding to HSV-hICAM1–transduced cells was compared with that to cells transduced with HSV not carrying the ICAM gene. Tumorigenicity of HSV-hICAM1–transduced tumor cells were tested in syngeneic Buffalo rats. Additionally, immunization with irradiated (10,000 rads) HSV-hICAM1–transduced tumor cells was performed to determine its effect on tumor growth.
Results: A 20-min exposure of tumor cells at a multiplicity of infection (MOI) of 1 resulted in high-level cell surface expression of human ICAM in approximately 25% of tumor cells. Transduced rat or human tumor cells exhibited significantly enhanced binding of lymphocytes ($p < 0.05$). HSV-hICAM1–transduced cells elicited an increase in infiltration by $CD4^+$ lymphocytes in vivo and exhibited decreased tumorigenicity. Immunization with irradiated HSV-hICAM1–transduced cells protected against growth of subsequent injected parental tumor cells.
Conclusions: HSV amplicon–mediated gene transfer is an efficient method for modifying the cell surface expression of adhesion molecules. Increased tumor expression of ICAM-1 represents a promising immune anti-cancer strategy.

Introduction

Cell–cell adhesion is central to an effective immune interaction between T cells and targets (1). Intercellular adhesion molecule-1 (ICAM-1) is a well-described adhesion molecule that mediates cell adhesion through a receptor on leukocytes known as leukocyte function antigen-1 (LFA-1) (2,3). Baseline in vivo expression of ICAM-1 is usually low and limited to endothelial cells, lymphoid cells and fibroblasts. ICAM-1 expression is up-regulated in inflammatory states in vivo and by

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interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) in vitro (4,5). There is mounting evidence that ICAM-1/LFA-1–mediated cell adhesion plays a critical role in the binding of T lymphocytes and may participate in immune activation. A series of studies have shown that monoclonal antibodies directed at ICAM-1 can inhibit cytotoxic T lymphocyte (CTL), natural killer (NK), and lymphokine activated killer (LAK) cell–mediated lysis of targets in vitro (6–9). Furthermore, transfection of the ICAM-1 gene into major histocompatibility complex (MHC)-deficient fibroblasts enhances stimulation of T cells (10). It has recently been postulated that the loss of ICAM-1 expression on the surface of tumor cells may be responsible for certain tumors' ability to evade the host immune system (11–13). Enhancement of expression of cell adhesion molecules such as ICAM-1, therefore, represents a potential strategy for therapeutic manipulation of anti-tumor immunity (14,15).

Viral vectors are among the most efficient means of transferring foreign genes into a cell. Herpes simplex viral (HSV) amplicon vectors are particularly suited for clinical gene therapy for a variety of reasons. HSV vectors rapidly and efficiently transduce both dividing and nondividing cells, allowing gene transfer into many cell types in as little as 20 min. Additionally, gene transfer with HSV vectors is episomal and gene expression is thus transient, which is desirable for cancer-related immunotherapy (16–20). Our previous studies have demonstrated the effectiveness of HSV vectors in transferring cytokine genes into tumor cells and eliciting significant antitumor activity by expression of these secreted proteins (16,17,21). The current studies examine the potential for HSV-mediated gene transfer in eliciting tumor cell surface production of the human ICAM-1 and the resultant immune consequences.

Materials and Methods

Tumor Cell Line

The tumor cell line Morris hepatoma McA-RH7777 (ATCC CRL 1601) was maintained in culture [Dulbecco's modified Eagle medium (DMEM), 6.25% fetal calf serum (FCS), 20% horse serum, 2 mM L-glutamine) and periodically implanted into Buffalo rat flanks to ensure tumorigenicity. The human colorectal tumor cell line HCT-8 (ATCC CCL 244) was maintained in culture (RPMI, 10% FCS) and passaged routinely. These cell lines were tested to be free of mycoplasma and viral infection.

HSV Vectors

Human ICAM-1 cDNA (purchased from R&D Systems, Minneapolis, MN) and *E. coli* β-galactosidase cDNA was directionally cloned into HSVPrPuc (HSV-hICAM1 and HSVlac, respectively), which contains the HSV immediate early 4/5 promoter, a multiple cloning site, and an SV40 A sequence, and packaged as previously described (18,19,21,22). Packaging of amplicon vectors was performed in RR1 cells using helper mutant D30EBA virus as previously described (21). Amplicon titers were determined by X-gal histochemical staining and by quantitative polymerase chain reaction (PCR) as described previously (21). Amplicon titer in the different virus preparations ranged from 1 to 10 $\times$ 10^7 pfu/ml and the helper titers were in the range of 5 to 15 $\times$ 10^7 pfu/ml. HSVlac titers were between 1 and 2 $\times$ 10^6 blue-forming units/ml as titered by expression and X-gal biochemistry on NIH3T3 cells. The HSV-hICAM1 titers were between 1 and 2 $\times$ 10^6 particles/ml.

In Vitro Production of ICAM-1

Hepatoma cells from culture were radiated with 10,000 rads and rested for 1 hr. Cells were then exposed to HSV-hICAM1, HSVlac, or nothing at an MOI of 1 for 20 min at 37°C. Cells were then washed with media twice and maintained in culture until analysis. To assess the cell surface expression of hICAM1, cells were harvested at 1, 2, 5, and 7 days after transduction and washed twice with Hank's buffered salt solution (HBSS) containing 10 mM HEPES. Separate aliquots of cells were then incubated on ice for 20 min with anti-human ICAM-1 (Clone MEM111, Caltag, Burlingame, CA) and anti-rat ICAM-1 (Clone 1A29, Caltag) antibodies conjugated to phycoerythrin (PE) or FITC. Additional aliquots of cells were incubated with isotype controls (Caltag) to account for nonspecific binding of antibodies. Cells were then analyzed with a FACscanner (Becton Dickinson, Franklin Lakes, NJ) for the presence of human and rat ICAM.

To analyze the secretion of soluble human ICAM (sICAM), radiated cells were transduced with HSV-hICAM1, HSVlac, or nothing and plated in 12-well plates. Culture supernatants were harvested at 1, 2, 5, and 7 days after transduction. Cells in representative wells were counted to standardize production to the num-

ber of cells per well. Supernatants were aliquoted and stored at −80°C until analysis. sICAM in the supernatants was quantitated by ELISA (HyCult Technologies, Uden, The Netherlands). The limit of detection for this assay was 100 pg/ml.

Adhesion Assay

RAT SPLENOCYTE ADHERENCE TO RAT HEPATOMA CELLS. To determine whether ICAM-1–transduced rat hepatoma cells bound lymphocytes more avidly, a modification of previously reported adhesion assays (23) was performed. Briefly, hepatoma cells were radiated with 10,000 rads, exposed to HSV-hICAM1, HSVlac, or nothing for 20 min at 37°C and washed with media twice. Cells were then plated in nearly confluent monolayers in 96-well plates. Splenocytes were harvested from normal Buffalo rats 1 day prior to each assay and cultured overnight in complete RPMI (.01 mM NEAA, 1 mM NaPyruvate, 2 mM L-glutamine, 50 μM 2-ME, penicillin/steptomycin) containing 10% FCS, 50 U/ml IL-2 (Chiron, Emeryville, CA), 5 μg/ml Con A (Sigma, St. Louis, MO), and 50 ng/ml PMA (phorbol 12-myristate 13-acetate) (Sigma). On the day of the assay, nonadherent splenocytes were harvested at a concentration of 10^6/cc, and labeled with MTT [5 mg/ml phosphate-buffered saline (PBS)] in a v:v ratio of 3:1 (splenocytes: MTT). Splenocytes were incubated with MTT for 6 hr at 37°C with gentle agitation every 30 min. Before the labeled splenocytes were added, one set of wells with hepatoma cells was incubated for 2 hr with anti-ICAM1 antibody (clone HA58, PharMingen, San Diego, CA). All wells were then washed once in media, and MTT-labeled splenocytes were then added at a concentration of 1×10^6/100 μl into each well. The cells were then coincubated for 30 min at 37°C. Nonadherent lymphocytes were gently washed off with PBS. Adherent lymphocytes were lysed with dimethylsulfoxide (DMSO) and read by spectrophotometry at 570 nm. Representative wells were used to count the number of hepatoma cells present for each experimental group. Additional labeled splenocytes were plated at varying concentrations, lysed, and read by spectrophotometry to create a standard curve for the number of splenocytes per well. An adhesion index calculated as the number of adherent lymphocytes per hepatoma target cell and the mean of 8 wells was recorded.

HUMAN LYMPHOCYTE ADHESION TO HUMAN COLORECTAL CANCER CELLS. Protocols were performed in compliance with Memorial Sloan-Kettering Cancer Center's Institutional Review Board guidelines. To determine if human colorectal cancer cells infected with HSV-hICAM1 increased lymphocyte adhesion, a modified adhesion assay was performed as above. Briefly, HCT-8 cells were irradiated with 9000 rads and then infected in suspension, with either HSV-hICAM1 or nothing (20 min, 37°C), and then washed twice with media. Cells were then plated at a concentration of 1×10^5 cells/well in 96-well plates.

Human lymphocytes were then harvested as previously described (24). Briefly, 15 cc of fresh heparinized blood was harvested from healthy human volunteers. The blood was mixed with an equal amount of PBS, and 9 cc of Ficoll-Paque (endotoxin tested, sterile; Amersham Pharmacia Biotech, Piscataway, NJ) was then layered beneath the mixture, and the gradient centrifuged at 2000 rpm, 20°C, for 30 min with no brake (Eppendorf, model 5810R). The monocellular layer was then harvested and washed twice in HBSS in a v:v ratio of 3:1. The cells were incubated overnight in complete RPMI (.01 mM NEAA, 1 mM NaPyruvate, 2 mM L-glutamine, 50 μM 2-ME, penicillin/streptomycin) containing 10% FCS, 50 U/ml IL-2 (R&D Systems), 5 μg/ml Con A (Sigma), and 50 ng/ml PMA.

Before MTT-labeled lymphocytes were added, one set of wells with HCT8 colorectal cancer cells was incubated for 2 hr with anti-ICAM1 antibody (clone HA58, PharMingen). The MTT-labeled lymphocytes and colorectal cancer cells were then coincubated (30 min, 37°C), washed, lysed, and read by spectrophotometry as stated above. The mean of 5 wells was recorded over 8 days at four different time points: days 1, 2, 5, and 8.

In Vitro Cell Proliferation

To determine whether transduction of hepatoma cells with the ICAM-1 gene altered in vitro growth properties, cell proliferation assays were performed. Replicating rat hepatoma cells were exposed to HSV-hICAM1, HSVlac, or nothing at an MOI of 1 for 20 min at 37°C. Cells were then plated in 24-well plates at a concentration of 10^4 viable cells/ml/well. Cells were harvested by trypsin disaggregation at 1, 2, and 4 days after plating and counted by trypan blue exclusion. The mean count of 8 wells per time point was compared.

Animals and Operative Procedures

Male Buffalo rats (Harlan Sprague Dawley) were housed 2 per cage in a temperature-(22°C) and humidity-controlled environment and were given water and standard rat chow (PMI Mills, St. Louis, MO) ad libitum. They were maintained in 12-hr light/dark cycles. All surgical procedures were carried out through a midline laparotomy under intraperitoneal (ip) pentobarbital (50 mg/kg) anesthesia. For major abdominal operations, 3 ml of 0.9% saline was administered ip for resuscitation postoperatively. All animals received care under approved protocols in compliance with Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee guidelines.

Tumorigenicity Experiments

To analyze the effects of ICAM-1 overexpression on the in vivo growth characteristics of hepatoma cells, flank tumorigenicity experiments were performed. Animals ($n = 5$ per group) were randomized to receive subcutaneous left flank injections of 10^6 viable rat hepatoma cells transduced with HSV-hICAM1, HSVlac, or nothing (MOI of 1). On the opposite right flank, all animals received subcutaneous flank injections of 10^6 viable nontransduced cells. Animals were weighed and tumors measured with external calipers twice weekly. Tumor measurements were made in two perpendicular dimensions and averaged. Tumor volume was calculated using the equation $4/3\pi r^3$.

Immunohistochemistry

To assess potential immunologic mechanisms of tumor regression, immunohistochemical analysis of cell infiltrates in tumors was carried out. Animals from additional tumorigenicity experiments had tumors excised at 1 and 3 weeks after injection of cells ($n = 5$ per time point) and placed immediately in 10% buffered formalin. Twenty-four hours later, tumors were embedded in paraffin through standard techniques, and 5-μm sections were made. Hematoxylin and eosin staining was performed through standard techniques. The following antibodies were used for immunohistochemical analysis: mouse monoclonal anti-rat CD4 (IgG1, clone W3/25, Serotec, Oxford, U.K.), mouse monoclonal anti-rat CD8 (IgG1, clone OX-8, Caltag), and mouse monoclonal anti-rat 1-A (IgG1, clone OX-6, Serotec), which recognizes rat MHC class II. The secondary antibody used was biotinylated anti-mouse IgG, rat adsorbed (Vector, Burlingame, CA). Slides used for CD4 and CD8 staining were pretreated with 1 mM EDTA (pH 8) in a microwave for 10 min. For MHC II staining, slides were pretreated for 10 min with a 0.05% protease XXIV (Sigma) in Tris-HCl buffer, pH 7.6. Endogenous peroxide was then quenched with a 5-min incubation in 3% H_2O_2. After washes with PBS, slides were placed in 0.05% bovine serum albumin (BSA) for 1 min. Slides were then dried and whole horse serum applied at a 1:20 dilution in 2% BSA and incubated for 10 min. Serum was suctioned off and 150 μl of primary antibody applied. The primary antibody was incubated for 16–18 hr at 4°C in a humidified chamber. After PBS washes, secondary antibody was applied to the slides at a 1:500 dilution in 1% BSA and incubated for 60 min at room temperature in a humidified chamber. Slides were then washed in PBS and peroxidase-conjugated streptavidin was applied at a dilution of 1:500 in 1% BSA. Slides were then washed with PBS and transferred to a bath of 0.06% diaminobenzidine (Sigma) for 5 to 15 min. Slides were washed in water and decolorized with 1% acid alcohol in water. Dehydration with ethanol and xylene were carried out with standard techniques and slides were mounted with Permount (Fisher, Pittsburgh, PA) mounting media.

A single pathologist blinded to the experiment reviewed the slides and graded them in the following way. Tumor cells were assessed for the presence or absence of MHC II staining. The degree of tumor infiltration with MHC II staining non-tumor cells was graded from 1 to 4. The degree of infiltration of tumors with the total amount of $CD4^+$ and $CD8^+$ lymphocytes was graded from 1 to 4. The relative percentage of $CD4^+$ and $CD8^+$ cells was then assessed and expressed as a ratio. Rat splenic tissue was used as a positive control for each experiment.

Vaccination Experiments

To determine whether previous exposure to ICAM-1–transduced hepatoma cells would protect against future challenges with the parental tumor, vaccination experiments were performed. Whole tumor cell vaccines were prepared as follows. Rat hepatoma cells were radiated with 10,000 rads, exposed to HSVhICAM1, HSVlac, or nothing at an MOI of 1 for 20 min at 37°C and washed twice with media. Animals ($n = 19$ per group) were then ran-

domized to receive either transduced cell type by intrasplenic injections of 10^6 cells in 200 μl of media on day 1. Control animals received 200 μl of media intrasplenically. Three weeks after vaccination, animals were challenged with 5×10^5 replicating hepatoma cells by intrasplenic injection. After 10 min, a splenectomy was performed in all animals. Three weeks after challenge, animals were sacrificed and liver surface tumor nodules counted. Body weights were recorded and grooming habits monitored twice a week throughout the experiment.

Statistics

Values are expressed as mean ± SEM. Values were compared by two-tailed Students *t*-test or ANOVA for multiple comparisons (SPSS software package, v. 7, Chicago, IL). *p* values of 0.05 or less were considered significant.

Results

In Vitro ICAM Production

With PE labeling, >90% of normal untreated rat hepatoma cells expressed rat ICAM on the cell surface with mean fluorescent intensities ranging from 200 to 288. There was no difference in rat ICAM expression between transduced and non-transduced cells. Cells transduced with HSVlac or no vector had no detectable surface human ICAM-1. Flow-cytometric analysis of rat hepatoma cells transduced with HSV-hICAM1 is illustrated in Figure 1. A 20-min exposure, at an MOI = 1, resulted in high-level expression of human ICAM on the surface of tumor cells. Peak cell surface positivity for human ICAM-1 was found 24 hr after transduction and tapered off by 1 week (the percentage of cells positive for hICAM1 was 25%, 16%, and 9% on days 1, 2, and 5 post-transduction). Mean fluorescent intensity of human ICAM-1 on HSV-hICAM1–transduced cells was 450, 271, and 124 on days

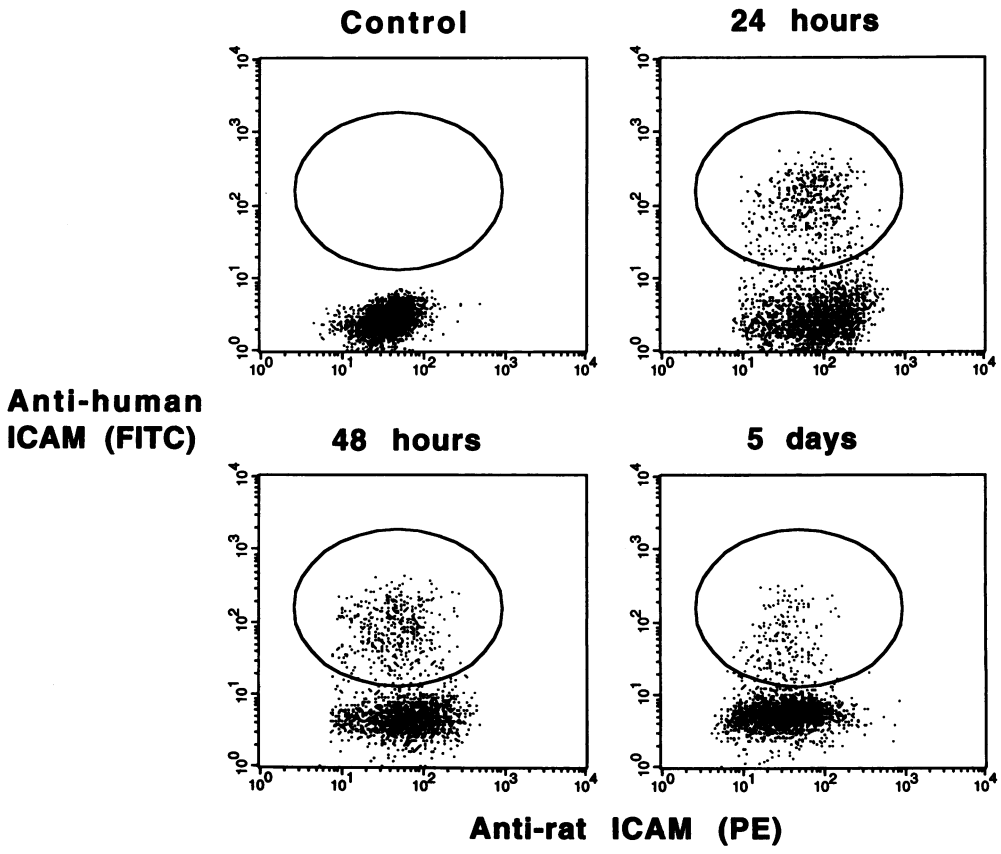

Fig. 1. Representative scattergrams of flow-cytometric results for human ICAM-1 expression in HSV-hICAM1-transduced Morris hepatoma cells at 1, 2, and 5 days after transduction.

1, 2, and 5, respectively. On day 7 post-transduction with HSV-hICAM1, only 4% of viable cells were positive for surface hICAM1.

Figure 2 illustrates the quantitation of soluble human ICAM found in cell culture supernatants of transduced cells. No soluble human ICAM was detectable in supernatants of cells transduced with HSVlac or nothing. Levels in supernatants of transduced cells peaked at 48 hr after transduction and approached the level of detection by day 7.

Adhesion

To determine whether hICAM1 gene transfer would alter rat lymphocyte binding by transduced tumor, an in vitro lymphocyte binding assay was used. Transduction by HSVlac or exposure of hepatoma cells to media did not alter lymphocyte binding (5517×10^3 lymphocytes/10^4 hepatoma targets). There was a significant ($p < 0.05$) increase in the number of adherent lymphocytes per hepatoma target cell in wells containing HSV-hICAM1–transduced cells (205 ± 15 at day one; 145 ± 10 at day two; 110 ± 12 at day five) compared to lac-transduced and untreated cells. Specific antibody to human ICAM-1 neutralized this increase in binding.

These findings were confirmed in experiments with human lymphocytes and human tumor. To determine whether hICAM1 gene transfer would enhance lymphocyte binding in human cells and to characterize these effects over time, an in vitro lymphocyte binding assay was carried out over 8 days. At 24 hr there was a significant increase in the number of adherent lymphocytes per colorectal cancer cell in wells containing HSV-hICAM1–infected cells compared to untreated cells ($p = 0.007$). Administration of anti-ICAM1 antibody eliminated this effect such that there was no difference between antibody-treated HSV-hICAM–transduced cells and nontransfected controls (Fig. 3).

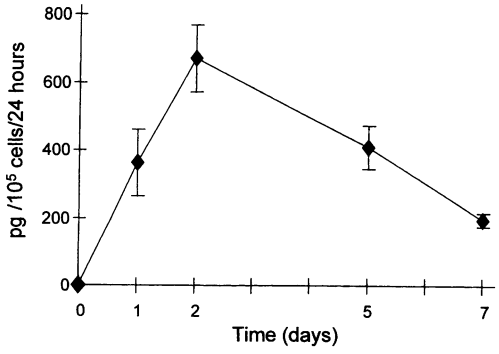

Fig. 2. Levels of soluble human ICAM by ELISA 1, 2, 5, and 7 days after transduction of radiated Morris hepatoma cells with HSV-hICAM1. Values expressed as mean ± SEM.

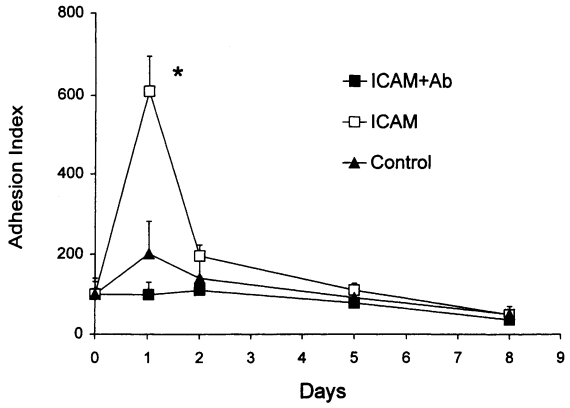

Fig. 3. Graphical representation of adhesion. Adhesion index over 8 days in human lymphocytes (no. of adherent lymphocytes × 10^3 per 10^5 human colorectal cancer target cell). Human colorectal HCT8 cells were transduced with HSV-hICAM1 (open squares), nontransduced controls (filled triangles), or incubated with antibodies to hICAM1 after transduction with HSV-hICAM1 prior to the adhesion assay (filled squares). *$p = 0.007$ versus controls and antibody-blocked cells.

In Vitro Cell Proliferation

Cells transduced with HSV-hICAM1 grew similarly in culture compared to HSVlac-transduced cells and untreated cells, indicating that changes in in vivo tumor growth (below) cannot be accounted for by changes in intrinsic growth rate of the modified tumor (Fig. 4).

In Vivo Tumorigenicity

There was significantly decreased tumor growth in the left flanks of animals injected with HSV-hICAM1–transduced cells compared to controls (Fig. 5). Tumor volumes at the termination of the experiment were compared. Tumors transduced with HSV-hICAM1 had a significantly ($p < 0.05$) smaller volume (1397 ± 1296 mm^3) than that of tumors transduced with HSVlac (7109 ± 2118 mm^3) and untreated tumors (13,556 ± 3354 mm^3). The slight reduction in tumor growth in animals treated with HSVlac likely reflects the trauma of the injections combined with the cytotoxicity of helper virus in the

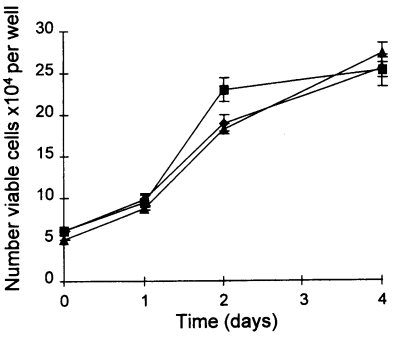

Fig. 4. In vitro proliferation of rat hepatoma cells transduced with HSV-hICAM1 (triangles), HSVlac (squares), or nothing (diamonds). Mean ± SEM of 8 wells per group per time point is shown. Experiments were repeated twice with similar results.

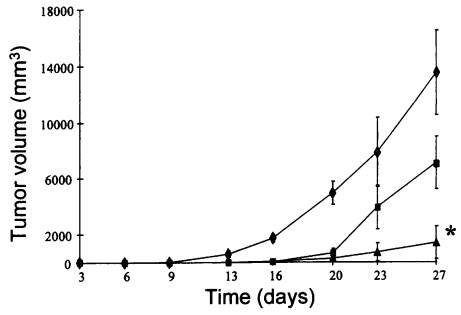

Fig. 5. Graphical representation of tumor growth in the left flank of rats. Rats (n = 5 per group) were injected with 10^6 viable hepatoma cells transduced with HSV-hICAM1 (triangles), HSVlac (squares), or nothing (diamonds). Values are expressed as mean ± SEM. *Three out of 5 animals failed to develop palpable tumors. Experiment was repeated once.

HSVlac preparation. On the contralateral untreated side, all groups had progressive tumor growth that was not significantly different.

Immunohistochemistry

The ratio of CD4 to total $CD4^+$ and $CD8^+$ T cells did not differ between groups at 1 week, but at 3 weeks, there was a significant increase in this ratio in the HSV-hICAM1–treated animals compared to HSVlac and untreated animals (0.42 vs. 0.25 and 0.24, $p < 0.05$). There was no significant difference in the degree of infiltration of tumors with MHC II staining immune cells between treatment groups at 1 and 3 weeks. Tumor cells did not stain positively for MHC II expression in any case.

Intrahepatic Vaccination

Throughout the experiment, there was no difference in weight gain among treatment groups and all animals maintained normal grooming habits. As illustrated in Figure 6, there was significantly decreased uptake and growth of hepatic metastases in animals vaccinated with HSV-hICAM1 cells compared to all controls ($p \leq 0.05$). There was no difference between animals vaccinated with HSVlac-transduced cells, radiated cells alone, or media.

Discussion

Stimulation of the host immune response against tumor antigens has shown great promise as an anticancer strategy. Systemic administration of cytokines such as IL-2 has shown efficacy in some patients with advanced malignancies, but use of IL-2 is limited by its significant toxicity (25,26). Gene therapy strategies utilizing genes coding for immunostimulatory cytokines such as IL-2, IL-4, IL-6, IL-7, TNF, IFN-γ and granulocyte-macrophage colony–stimuling factor (GM-CSF) (27–37) have shown promise in enhancing the host anti-tumor immune response in animal models. The expression of cell surface proteins that may bind and/or stimulate T lymphocytes and enhance antigen presentation represents another potential immunostimulatory anti-tumor

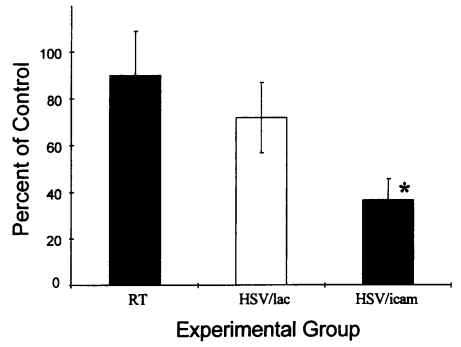

Fig. 6. Number of surface tumor nodules in rat livers. Rat livers (n = 19 per group) after challenge with 5×10^5 viable hepatoma cells. Three weeks prior to challenge, animals underwent intraportal vaccination with media (control), 10^6 radiated cells alone (solid bar), 10^6 radiated cells transduced with HSVlac (empty bar), or 10^6 radiated cells transduced with HSV-hICAM1 (gray bar). *$p \leq 0.05$ versus all controls.

strategy. The costimulatory B7 molecules are cell surface proteins that have been used successfully in experimental models (38–40) and are now under evaluation in human clinical trials (41). The use of tumor cells engineered to constitutively express the adhesion molecule ICAM-1 represents another novel approach for enhancing anti-tumor immunity in vivo.

The role and clinical significance of ICAM-1 in cancer are multifold. It is clear that many malignant cell types express ICAM-1 on its cell surface (42). In murine models, the level of soluble ICAM-1 shed from melanoma was found to correlate with tumor burden (43). Expression and overexpression of ICAM-1 on the tumor cells can potentially have beneficial as well as detrimental effects. Tumor cell surface ICAM-1 expression has been postulated to have negative effects in promoting biologic activities important in the metastatic process, including tumor transport, endothelial cell adhesion, and angiogenesis (44,45). However, there is a growing body of literature that indicates the alternative: that ICAM-1 overexpression may improve host tumor surveillance. ICAM-1 cell surface expression is critical for lymphocyte adhesion to antigen-presenting cells (APCs) and for T cell–mediated cytolysis (1,3). Monoclonal antibody experiments have shown that the loss of ICAM-1/LFA-1–mediated adhesion can prevent T cell adhesion and cytolysis of targets (6–9). Cellular transfection experiments have demonstrated that interaction between ICAM-1 and the LFA-1 receptor is critical for effective HLA class II–restricted and allospecific T cell activation (10). Thus, there is much evidence that ICAM-1 is involved in activation of cellular immunity. The current study demonstrates that expression of cell surface ICAM-1 can be enhanced by transduction with the hICAM1 gene through HSV vectors and result in increased adhesion of lymphocytes in a specific manner that can be blocked by antibodies to hICAM-1.

Attempts have therefore been made to modulate tumor surveillance in animal models by enhancement of ICAM-1 expression. Wei et al. (15) examined effects of transducing rat ICAM or human ICAM into rat tumor cells. Tumorigenicity was significantly decreased with transfection with constructs coding either rat or human ICAM-1. The authors found that vaccination with rat tumor cells expressing human ICAM-1 also attenuated growth of established tumors in a model of peritoneal metastases in Fisher rats, whereas vaccination with tumor cells expressing rat ICAM-1 had no demonstrable effect. Wei et al. concluded that these findings for tumor cells transfected with human ICAM-1 gene were due in part to the xenogeneic nature of the human ICAM-1 protein in their rat model. While this is probably one reason why the human ICAM-1 expression proved more efficacious than expression of rat ICAM-1, there are many possible explanations for vaccination with rat ICAM-1 cells having no effect on outcome in their models of established tumor. It is likely that the experimental protocol of vaccination on day 9 after peritoneal injection of parental tumor cells, a time when animals have already begun to die, is too severe a model. In addition, it is possible that lymphocyte binding without other stimulatory signals may be insufficient for effective anti-tumor effector actions. There is abundant data in other models to show that accentuated expression of syngeneic ICAM-1 will produce potent anti-tumor effects. Treatment of human melanoma and colon cancer cell lines with IFN-γ increases expression of ICAM-1 and increases vulnerability of these cells to macrophage-mediated killing (46). Such increased susceptibility is specific and can be blocked by anti-ICAM-1 antibodies. ICAM-1 gene transfer has also been used successfully to increase host surveillance for experimental tumors. Sartor et al. (14) transfected a murine fibrosarcoma cell line with the gene encoding the murine ICAM-1 and selected out clones of ICAM-expressing cells. Tumorigenicity of expressing clones was significantly decreased, though no assessment of long-term immunity was performed. Uzendoski et al. utilized a recombinant vaccinia virus expressing murine ICAM-1 to produce induction of anti-tumor response (47). MC38 murine colon carcinoma cells infected with this vaccinia virus had decreased tumorigenicity. Vaccination with such modified tumor cells protected against subsequent tumor challenge.

Thus, there is a good biologic basis to support investigation of ICAM-1 gene transfer as immunomodulation in cancer therapy. Ultimately, however, human testing is necessary to determine utility of this approach; what is needed is a clinically useful delivery system for the human ICAM-1 gene. In this regard, our work adds significantly to these previously published studies in providing a clinically relevant system for testing the utility of ICAM-1 gene therapy in humans. In the present study, tumor cells were subjected to a single 20-min exposure to HSV vectors and tested for biological activity without

selection for only the human ICAM-expressing cells. Despite the fact that only a percentage of cells was transduced, we were still able to induce significant binding of lymphocytes and to elicit an immunological reaction. We are able to reproduce all of the effects of ICAM-1 gene transfer seen in previous works by Wei et al. (15) without the time-consuming process of cell selection. Whether similar biologic effects can be seen in vivo and will be clinically useful awaits trials in humans.

HSV vectors are an excellent vector for use in gene therapy directed at cancer. This report extends prior findings from our laboratories and others (16,17,19,21,48) that HSV vectors are a rapid and efficient means of transferring genes of interest into dividing or nondividing cells. Transduction of tumor cells can be accomplished efficiently in 20 min and expression is relatively high for at least 1 week. A recent publication from our laboratories has demonstrated the feasibility of harvesting fresh tumor samples at operation, followed by radiation and successful transduction with HSV vectors to elicit high local production of secreted proteins such as IL-2 or GM-CSF (22). The current study indicates that HSV-mediated gene transfer may also lead to high-level expression of cell surface proteins. Given the rapidity and efficiency of HSV-mediated gene transfer, a clinical vaccination protocol using these vectors appears imminently feasible.

Initial human trials using systemic immunostimulatory cytokines were done on patients who had extensive tumor burdens and no other treatment options. While some responses to immunotherapy were noted, complete responses were rare (49,50). Immunotherapy utilizing gene therapy for the treatment of cancer will likely be most effective in the treatment of microscopic or minimally residual tumor. Hepatic malignancy is a good model of microscopic residual disease because in most patients who undergo resection of tumor, disease recurs in the remnant liver, thus implicating residual microscopic hepatic disease at the time of operation as the cause for therapeutic failures. We investigated an intraportal vaccination strategy with hepatoma cells transduced with the human ICAM-1 gene and have demonstrated that this strategy can decrease growth of microscopic disease in the liver. In a similar rat hepatoma model, we have shown that by combining immunotherapy targeting macrophage activity with INF-γ and lymphocyte activity with IL-2 or GM-CSF, we can stimulate more effective anti-tumor immunity than either modality alone (21). It is likely that a clinically relevant, immunostimulatory anti-cancer strategy will consist of multitargeted therapy. Some combination of tumor-expressed genes, including chemokines to attract immune cells to the tumor target, cell adhesion molecules to bind immune cells, and cytokines to activate the immune cells, holds promise. This report contributes to the evidence that the up-regulation of cell adhesion molecules using gene transfer strategies is a potentially useful method of binding immune cells at sites of tumor. These studies indicate that ICAM-1 is a therapeutic target and encourages studies combining induction of cell surface ICAM expression with other immunostimulatory strategies.

Acknowledgments

This work was supported in part by grants CA76416 and CA72632 from the National Institutes of Health and from the Sara Chait Foundation.

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