A Tumor-Associated Glycoprotein That Blocks MHC Class II-Dependent Antigen Presentation by Dendritic Cells

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A Tumor-Associated Glycoprotein That Blocks MHC Class II-Dependent Antigen Presentation by Dendritic Cells

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Tumors evade immune surveillance despite the frequent expression of tumor-associated Ags (TAA). Tumor cells escape recognition by CD8+ T cells through several mechanisms, including down-regulation of MHC class I molecules and associated Ag-processing machinery. However, although it is well accepted that optimal anti-tumor immune responses require tumor-reactive CD4+ T cells, few studies have addressed how tumors evade CD4+ T cell recognition. In this study, we show that a common TAA, GA733-2, and its murine orthologue, mouse epithelial glycoprotein (mEGP), function in blocking MHC class II-restricted Ag presentation by dendritic cells. GA733-2 is a common TAA that is expressed normally at low levels by some epithelial tissues and a subset of dendritic cells, but at high levels on colon, breast, lung, and some nonepithelial tumors. We show that ectopic expression of mEGP or GA733-2, respectively, in dendritic cells derived from murine bone marrow or human monocytes results in a dose-dependent inability to stimulate proliferation of Ag-specific or alloreactive CD4+ T cells. Dendritic cells exposed to cell debris from tumors expressing mEGP are similarly compromised. Furthermore, mice immunized with dendritic cells expressing mEGP from a recombinant adenovirus vector exhibited a muted anti-adenovirus immune response. The inhibitory effect of mEGP was not due to down-regulation of functional MHC class II molecules or active suppression of T cells, and did not extend to T cell responses to superantigen. These results demonstrate a novel mechanism by which tumors may evade CD4+ T cell-dependent immune responses through expression of a TAA.
Interference with TAA uptake, consequent activation, Ag processing, or cell surface Ag presentation properties on the APC could potentially result in inhibition of an anti-tumor immune response.

Our interest in developing active vaccination strategies for colon cancer led to studies of an important human TAA, GA733-2 (18), and its murine homologue mouse epithelial glycoprotein (mEGP) (19). GA733-2 (also known as Trop-1, KSA, CO17-1A, Ep-Cam, EG3P314) is a type I integral membrane glycoprotein that is expressed on the surface of a variety of normal epithelial cells and is overexpressed on many human carcinomas, particularly of the breast, colon, and lung (20–22). When expressed ectopically in fibroblasts or epithelial cells, GA733-2 has been shown to mediate homotypic cell-cell interactions and to interfere with adhesion mediated by conventional adhesion molecules (23, 24), but its physiological function is not known. mEGP bears 81% amino acid identity to GA733-2, has a similar expression profile in normal tissues (19, 25), and is similarly overexpressed in some murine tumor cell lines. As part of an effort to establish a murine model for anti-carcinoma immune responses, we attempted to immunize mice with DC-expressing mEGP. Surprisingly, we found that bone marrow-derived DC transduced to express mEGP lost their ability to stimulate CD4 T cells. We show here that mEGP specifically interferes with Ag presentation by MHC class II molecules on DC, thereby blocking CD4 T cell stimulation. Our results reveal a novel mechanism by which a TAA can facilitate evasion of anti-tumor immune responses.

Materials and Methods

Mouse bone marrow-derived DC culture

Bone marrow DCs were obtained from the indicated strains of mice and cultured as described (26, 27). Briefly, bone marrow cells were cultured in RPMI 1640 containing 10% FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 1000 U/ml GM-CSF (R&D Systems, Minneapolis, MN). In some experiments, 1000 U/ml IL-4 (R&D Systems) with or without 1.5 μg/ml indomethacin (Cayman Chemical, Ann Arbor, MI), and 50 μM NG-monomethyl-L-arginine (Calbiochem, San Diego, CA) were also added. Cells were washed with PBS and supplied with fresh medium on days 3 and 5 of culture. On day 7, non–adherent cells were harvested and infected with recombinant adenovectors at a multiplicity of infection (MOI) of 100 PFU at 37°C for 2 h. Control cells were uninfected. Cells were cultured for an additional 48 h in the presence of LP5 (serotype 026:B6, 200 ng/ml; Sigma-Aldrich, St. Louis, MO) or TNF-α (200 U/ml; R&D Systems). The cells were then characterized by expression of surface molecules, used as stimulators in functional assays, and/or used for in vivo administration.

Mice

Female BALB/c, C3H, and C57BL/6 mice (6- to 8-wk-old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Transgenic mice expressing the D011.10 and 3A9 T cell receptors were kindly provided by Drs. L. Turka and R. Eisenberg (University of Pennsylvania, Philadelphia, PA), respectively. Transgenic mice expressing the OTII (28) and OTI (29) TCRs, bred to Thy1.1 congenics and used at 6–12 wk of age, were generous gift of Drs. C. Surh (The Scripps Research Institute, La Jolla CA) and H. Shen (University of Pennsylvania), respectively.

Flow cytometry

Cells were resuspended to 5 × 10^6 cells/ml in RPMI 1640 with 3% FBS, 0.15 M HEPES, and 0.1% sodium azide, and preincubated according to the manufacturer’s instructions (BD Pharmingen, San Diego, CA) with mAbs to CD16 and CD32, specific for FcγRII and RI, to prevent nonspecific staining. Cells were then stained with saturating concentrations of the indicated Abs for 30 min at 4°C, washed, and analyzed on a FACSscan (BD Biosciences, Mountain View, CA) using CellQuest software version 3.1. The following Abs and isotype controls, directly conjugated to either FITC, PE, or PerCP were purchased from BD Pharmingen: anti-MHC class I (H-2D^d; clone 34-2-12), anti-MHC class II (I-A^d/E^d; clone 209; F-A^d, clone 11-5-2), anti-CD80/85-7 (clone 16-10A1), anti-CD86/87-2 (clone GL1), anti-CD11c (clone HL3), anti-CD11b (clone M1/70), anti-CD40 (clone 3/23), anti-CD48 (clone HM48-1), anti-CD54/CAM-1 (clone 3E2), anti-VJ5 (clone MR9-4), and anti-Thy1.1. mAbs G8.48 recognizing mEGP and anti-GA733-2 were purified from supernatants of the hybridomas (kindly provided by Dr. D. Herlyn, Wistar Institute, Philadelphia, PA), and FITC-conjugated (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions. Dilution of CFSE for OTI and OTII T cells was quantified by flow cytometry using VJ5-PE-positive and Thy1.1-PerCP-positive populations.

Construction of adenoviral vectors

mEGP cDNA was provided by Dr. W. M. Kuehl (National Cancer Institute, Bethesda, MD). GA733-2 cDNA was a gift of Dr. D. Herlyn (Wistar Institute). cDNAs were subcloned into pAdCMVlink, which was used to prepare E1 and E3-deleted adenoviruses (Ad.mEGP and Ad.GA733, respectively) as previously described (30). An adenoviral vector without transgene (Ad.Bgl; kind gift of Dr. J. M. Wilson, University of Pennsylvania) served as control.

Mixed lymphocyte reaction (MLR)

Two days after transduction, DC were treated with 100 μg/ml mitomycin C (Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C and used in different concentrations as stimulators for allogeneic or syngeneic T cells (1 × 10^5/well). T cells were isolated from splenocytes by negative selection (MACS) and cell enrichment columns (R&D Systems), and were >99% pure as determined by anti-Thy-1.2 staining (Ab clone 53-2.1; BD Pharmingen). After 4 days of culture, 1 μCi of [3H]thymidine (NEN Life Science, Boston, MA) was added per well. After 16 h the cells were harvested using a Tomtec Mach IIIIR harvester (Harmand, CT) and the incorporated [3H]thymidine was measured by scintillation counting using a Microbeta TriLux (PerkinElmer, Gaithersburg, MD). Con A was purchased from Boehringer Mannheim (Indianopolis, IN). Anti-CD3-Ab was obtained from BD Pharmingen (clone HIT3a).

ELISA

The following Ab pairs were purchased from BD Pharmingen and used according to the manufacturer’s instructions: IL-2, clones JES6-1A12 and JES6-3H8; IFN-γ, clones R4-6A2 and XMG1.2; IL-10, clones JES5-2A5 and JES5-2D11; IL-4, clones 11B11 and JES5-16E1; TNF-α, clones R4-6A2 and JES5-2D11. All ELISAs had a detection limit of 60 pg/ml. ELISAs were developed using HRP-conjugated secondary Abs and trimethylbenzidine substrate (BD Pharmingen) according to standard protocols. OD readings were performed on a Bio-Rad Microplate Reader (model 3550; Hercules, CA). ELISAs of OTI and OTII T cell supernatants were done using biotin-conjugated anti-IFN-γ or anti-IL-2 Abs (BD Pharmingen), and developed using streptavidin-conjugated alkaline phosphatase (Chemicon International, Temecula, CA) and 1 μg/ml p-nitrophenyl phosphate (Sigma-Aldrich) as a substrate. The plates were read at 405–650 nm on a Molecular Devices (Sunnyvale, CA) microplate reader.

Ag-specific T cell proliferation assays

T cells from 3A9 or D011.10 transgenic mice were purified from splenocytes using negative selection as described above for MLR. T cells recognizing hen egg lysozyme (HEL) 46–61 peptide presented by F-A^d, were stimulated with DC from C3H mice pulsed with either HEL protein (Sigma-Aldrich) or HEL46–61 peptide (Protein Sciences Facility, University of Illinois, Urbana-Champaign, IL). D011.10 T cells, recognizing OV323–339 peptide presented by F-A^d, were stimulated with DC from BALB/c mice pulsed with either OVA protein (Sigma-Aldrich) or OVA323–339 peptide (Cancer Center Peptide Synthesis Core, University of Pennsylvania). The pulse was done with 0.5 mg/ml protein for 16 h or with 1 μg/ml peptide for 2 h. DC were washed extensively and treated with mitomycin C (100 μg/ml for 30 min), then cocultured with the Ag-specific T cells as in the MLR. Supernatant responses were conducted in the same manner with the addition of 10 ng/ml staphylococcal enterotoxin B (SEB; Sigma-Aldrich) before incubation with T cells for 3 days.

CD4 T cells from OTII transgenic mice and CD8 T cells from OTI transgenic mice were purified by positive selection using AUTOMACS columns (Miltenyi Biotec, Auburn, CA), and were 94–98% pure as determined by FACS analysis using a VJ5-specific Ab. Cells were labeled with CFSE (Molecular Probes, Eugene, OR) as described (31, 32). For stimulation of OTI and OTII T cells, a mixture of 3 μg/ml OVA323–339 peptide (for OTII) and 1 μg/ml SIINFEKL peptide (for OTI) was added to cultures of uninfected or infected DC from C57BL/6 mice on day 7; controls had no peptide. Cells were washed three times on day 9 before culture with 100,000 CFSE-labeled T cells/well in 96-well plates (for proliferation studies) or 300,000 T cells/well in 48-well plates (for cytokine production) for 4 days. Quantification of CFSE dilutions was done as described (31, 32).
Responder frequency is the proportion of the initial pool that commits to division, and proliferative capacity is the average number of divisions that each responding cell undergoes (31).

**T cell hybridomas**

T cell hybridomas RF3370 and M22D9 (kindly provided by Dr. K. L. Rock, University of Massachusetts, Worcester, MA) recognize OVA peptides presented by MHC class I (H-2Kk, RF3370) or MHC class II (I-Aß, M22D9). DC from C57BL/6 mice were used as stimulators and pulsed with OVA protein (0.5 mg/ml for 16 h). Hybridoma cells (1 x 10^5) were cocultured with 2 x 10^6 DC for 24 h, then culture supernatant was harvested and assayed for IL-2 concentration by ELISA.

**Cell debris assay**

The naturally mEGP-negative FBL-3 murine erythroleukemia cell line (kindly provided by Dr. W. Chen, Cleveland Clinic Foundation, Cleveland, OH) was infected with Ad.mEGP or Ad.Bgl as for DCs. Forty-eight hours after transduction, cells were subjected to three freeze-thaw cycles to generate debris. Protein was quantitated using the Bio-Rad Protein DC assay. DC were incubated with increasing amounts of the cell debris for 16 h, and then the DC were washed extensively and used as stimulators in an allogeneic MLR.

**In vivo immunization and assay for adenovirus response**

BALB/c mice were injected i.v. with 10^7 untransduced DC (no virus) or DC that had been transduced 48 h earlier with Ad.mEGP or Ad.Bgl. To monitor anti-adenovirus T cell responses, 5 x 10^5 splenocytes harvested from immunized mice after 7 days were cultured with 10^6 DC that had been transduced 48 h earlier with Ad.Bgl. After 48 h in culture, 1 μCi of [3H]thymidine was added and cultures continued for an additional 16 h. Cells were then harvested and [3H]thymidine uptake was quantitated by scintillation counting. To monitor anti-adenovirus Ab responses, mice were euthanized after 7 days postinfection, and sera were collected. Serial dilutions of sera were assayed for adenovirus-specific IgM, IgG1, IgG2a, and IgG2b Abs by ELISA as described above using plates coated overnight with heat-denatured Ad.LacZ virus (5 x 10^9 particles/well) and secondary isotype-specific HRP-conjugated Abs (BD Pharmingen).

**Human DC and CD4+ T cell cultures**

Peripheral blood was obtained from healthy donors after informed consent under an Institutional Review Board-approved protocol. PBMCs were separated by density gradient centrifugation of 45 ml of heparinized whole blood using lymphocyte separation medium (BioWhittaker/Cambrex, East Rutherford, NJ). CD4+ T cells were separated from PBMCs and frozen as described (33). In parallel, DCs were generated as described (34). Briefly, PBMCs were plated in AIM-V medium (In Vitrogen Life Technologies, Carlsbad, CA) for 2 h at 37°C. Nonadherent cells were removed, and adherent cells were cultured with 800 IU/ml GM-CSF (Immunex, Seattle, WA) and 500 IU/ml IL-4 (R&D Systems) for 7 days. On day 7, immature DCs were harvested, counted by trypan blue exclusion, and transduced for 2 h at 37°C with Ad.GA733 or Ad.Bgl at 10^9 particles per cell. Transduced immature DCs were replated at 5 x 10^5 cells/ml in AIM-V medium supplemented with 1000 IU/ml GM-CSF, 1000 IU/ml IL-4, and 1 μg/ml LPS for 48 h. DCs were then harvested, counted by trypan blue exclusion, evaluated for their size on a Coulter counter (Beckman Coulter, Miami, FL), evaluated for GA733-2 expression by flow cytometry, and used for phenotypic and functional analysis. All reagents (except LPS) and media were endotoxin-free as determined by a quantitative chromogenic Limulus amebocyte lysate assay (BioWhittaker/Cambrex).

**Human DC functional assays**

For allogeneic MLR, DCs were irradiated (30 Gy from a 137Cs source) and seeded in triplicate at varying numbers with 10^5 thawed, washed CD4+ T cells from allogeneic healthy donors in AIM-V supplemented with 3% heat-inactivated human AB serum (BioWhittaker/Cambrex). For autologous MLR, CD4+ T cells were from the same healthy donor from whom the DCs were generated. Proliferation was measured 6 days later by incorporation of [3H]thymidine (1 μCi/well) added for the last 18 h of culture. Plates were harvested and thymidine incorporation was measured by scintillation counting. Dose-response T cell proliferation assays were also performed at a DC:T cell ratio of 1:10, using DC transduced with Ad.GA733 or Ad.Bgl at 10^9 particles per cell, irradiated, and pulsed with SEB at 0.01–1000 ng/ml.

**Results**

Expression of mEGP impairs the ability of DC to stimulate an allogeneic T cell response

Bone marrow-derived mouse DC do not express mEGP as determined by RT-PCR (data not shown) and flow cytometry (Fig. 1) (35). To investigate the function of mEGP, murine DC were transduced with a control replication-deficient adenoviral...
vector (Ad.Bgl) or with vectors encoding mEGP (Ad.mEGP) or GA733-2 (Ad.GA733). Expression of mEGP or GA733-2 at the surface of Ad.mEGP and Ad.GA733 transduced cells, respectively, was verified by flow cytometry (Fig. 1). Flow cytometric analysis 2 days postinfection failed to detect any significant differences among uninfected cells and cells infected with Ad.mEGP, Ad.GA733, or control virus (Ad.Bgl, not shown) with respect to cell surface expression of MHC class I, MHC class II, B7-1 and B7-2, CD11b, CD11c, CD40, CD48, and CD54 (Fig. 1).

Interestingly, despite the lack of any detectable effect on MHC class II cell surface expression, murine DC expressing mEGP showed a marked decrease in the ability to stimulate purified CD4+ allogeneic T cells in MLR, relative to uninfected DC or DC expressing the human homologue, GA733-2 (Fig. 2A). The defect in T cell proliferation was accompanied by a significant decrease in production of IL-2 (Fig. 2B) and IFN-γ (Fig. 2C). IL-4 production was not detectable in these assays, and IL-10 production was similar for all groups (100–200 pg/ml in supernatants). Similar results were obtained using infected DC from BALB/c, C3H, or C57BL/6 mice as stimulators and T cells from C3H or BALB/c mice as responders. The MLR inhibitory effect of mEGP was observed when DC matured for 48 h with LPS (200 ng/ml; Fig. 2A) or with TNF-α (200 U/ml; not shown). Despite the striking difference in T cell stimulatory capacity, there was no consistent difference in the viability of mEGP and GA733-2 transduced DC in culture over a 5-day period (not shown).

To determine whether mEGP expression affected T cells or DCs in the MLR, we assessed the effect of adding a second TCR stimulus. Addition of a second T cell stimulus restores the MLR proliferative response in the presence of mEGP (Fig. 2E). These data show that T cells exposed to mEGP-expressing DC are capable of proliferating if provided with an appropriate TCR stimulus, and are therefore not tolerized or inactivated. They thus suggest that mEGP expression may result in a failure of the DC to stimulate T cells through the MHC-TCR interaction. This possibility is examined in more detail below.

**The effect of mEGP is dose-dependent and limited to the cells on which it is expressed**

Increasing doses of Ad.mEGP during infection of DC (increasing the MOI) led to greater levels of mEGP expression, as assessed by flow cytometry (not shown), with a proportional decrement in T cell stimulation (Fig. 3A). This shows that the degree of inhibition of T cell stimulation is dependent upon the level of mEGP expression. Moreover, cocultures of control DC with DC uniformly expressing high levels of mEGP led to T cell responses that were directly proportional to the number of control DC in the well (Fig. 3B). The mEGP-expressing cells failed to inhibit the response to control cells throughout the dilution range, even when present at a 4-fold excess. These data thus suggest that mEGP acts at the level of the individual DC and does not exhibit a trans-dominant negative inhibition of neighboring DC. Consistent with this observation, conditioned media from mEGP-expressing cells failed to inhibit the T cell response (not shown).

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** DCs transduced to express mEGP fail to stimulate a MLR but support superantigen responses. A–C, Untransduced BALB/c bone marrow-derived DC (BMDC), DC transduced with Ad.Bgl, Ad.mEGP, or Ad.GA733, or negative control NIH 3T3 cells were used as stimulators in a 5-day MLR with CD4+ T cells from C57BL/6 mice. A, T cell proliferation was measured by [3H]thymidine uptake during the final 16 h. B and C, Culture supernatants were assayed for IL-2 (B) or IFN-γ (C) production by T cells. Expression of mEGP, but not GA733-2, impairs the ability of DC to stimulate T cells in all three assays. D, T cells were incubated for 5 days alone or with DC transduced with Ad.Bgl or Ad.mEGP in the absence or presence of Con A or soluble anti-CD3-Ab as a second T cell stimulus. Addition of a second T cell stimulus restores the MLR proliferative response in the presence of mEGP. E, Untransduced DC from BALB/c mice or DC transduced with Ad.Bgl or Ad.mEGP were mixed with 10^5 autologous CD4+ T cells in the absence or presence of 10 ng/ml SEB, and proliferation was measured on day 3 by [3H]thymidine uptake.
Expression of mEGP impairs the ability of DC to stimulate a MHC class II-dependent, Ag-specific T cell response

To determine whether the inhibitory effect of mEGP could be observed using specific antigenic stimuli, we tested whether mEGP expression altered the ability of Ag-pulsed DC to stimulate Ag-specific T cells. CD4⁺ T cells were isolated from transgenic mice expressing either the DO11.10 TCR specific for OVA peptide 323–339/I-A^d (36) or the 3A9 TCR specific for HEL peptide 46–61/I-A^b (37). The T cells were stimulated with transduced or untransduced DC pulsed with either the respective full-length protein or the antigenic peptide. The stimulatory capacity of DC pulsed with either protein or peptide was markedly reduced by mEGP expression, with no apparent dominant effect by mEGP-expressing cells.

Because the DO11.10 (OVA), OTII (OVA), and the 3A9 (HEL) TCRs are all restricted by MHC class II molecules, we sought to determine whether mEGP expression also inhibited Ag presentation by MHC class I molecules. To this end, peptide-pulsed DC resulted in [³H]thymidine incorporation below 1000 cpm in both models (not shown). E and F, Varying numbers of untransduced or virally transduced DC from C57BL/6 mice were pulsed with OVA323–339 and SIINFEKL peptides and incubated with CFSE-labeled CD4⁺ T cells isolated from transgenic OTII mice; these T cells express a TCR specific for OVA323–339 peptide presented by I-A^d. After 4 days, cells were analyzed by flow cytometry, and CFSE labeling of Vβ⁺ T cells was quantitated and analyzed for proliferative capacity (E) and responder frequency (F).

Values for proliferative capacity and precursor frequency were determined as described (31, 32). As shown in Fig. 4, E and F, both the precursor frequency and the proliferative capacity of responding T cells were decreased in response to mEGP-expressing DC relative to control DC. Proliferative capacity has been linked to the duration of TCR engagement, whereas responder frequency is related to the quantity of Ag display (38). Thus, Ag presentation is both qualitatively and quantitatively reduced by mEGP expression.
Expression of mEGP does not affect the ability of DCs to stimulate MHC class I-restricted CD8^+ T cell responses. Varying numbers of untransduced or virally transduced DC from C57BL/6 mice were pulsed with OVA323–339 and SIINFEKL peptides. A and B. Cells were then incubated with CD8^+ T cells isolated from transgenic OT1 mice; these T cells express a TCR specific for OVA257–264 peptide (SIINFEKL) presented by H-2K^b. After 4 days, supernatants were harvested and assayed by ELISA for IL-2 (A) and IFN-γ (B). Results are representative of two experiments. Note that all cells showed similar proliferation profiles that were independent of the presence of DCs but dependent on the presence of SIINFEKL peptide. DC transduced with Ad.Bgl elicited similar or higher levels of IL-2 and IFN-γ at all doses (not shown). C. The same peptide-pulsed DC were incubated with CFSE-labeled CD4^+ T cells isolated from OTIi mice, and 4 days later Vβ5^+ cells were analyzed for CFSE dilution by flow cytometry. Shown are representative profiles from an experiment in which the number of DC per well is indicated in the upper right corner.

Expression of GA733-2 inhibits Ag presentation by human DCs

Given their high sequence homology, it was surprising that expression of mEGP in mouse DCs inhibited Ag presentation whereas expression of the highly homologous GA733-2 did not. To determine whether this reflected species restriction by DCs on the inhibitory effects of this protein, we tested whether GA733-2 expression affected Ag presentation by human DCs. Human DCs derived from peripheral blood of healthy donors were transduced with either Ad.GA733 or Ad.Bgl, and then tested for their ability to stimulate primary CD4^+ T cells isolated from the same or different donors. By analogy to the effects of mEGP on mouse DCs, expression of GA733-2 by human DCs potently inhibited their ability to stimulate T cells in an allogeneic MLR, whereas infection with the control Ad.Bgl resulted in enhanced T cell stimulation (Fig. 6A). The enhancement by Ad.Bgl was likely due to stimulation of adenosivirus-specific T cells, because 1) the donors were likely immune to adenoviruses and 2) these same DCs stimulated a vigorous proliferative response from autologous T cells (Fig. 6C). Expression of GA733-2 did not inhibit the ability of human DCs to stimulate T cells in the presence of superantigen (SEB; Fig. 6B). As with mEGP expression in mouse DCs, GA733-2 expression did not inhibit the ability of human DCs to stimulate T cells in the presence of superantigen (SEB; Fig. 6C). Thus, the mouse and human orthologues of GA733-2 both inhibit Ag presentation by DCs, but display species restriction in their inhibitory capacity.

Impaired stimulation of MLR by DC pulsed with cell debris from tumors expressing mEGP

By virtue of their high capacity for macrophagy of necrotic and apoptotic cells, DC within the tumor microenvironment are able to engulf tumour cell debris and process the resident proteins for presentation by MHC class I or class II molecules (40–42). Given the observed inhibition of Ag presentation by MHC class II molecules in DC that express mEGP, we postulated that the presence of mEGP in internalized tumor cell debris might also inhibit T cell proliferation. DC were exposed to homogenates of a mEGP-nonexpressing cell line, FBL-3, that had been transduced with control adenovirus or
Ad.mEGP. The expression of mEGP on Ad.mEGP transduced cells was 2–3 logs over background (data not shown), comparable to the level of GA733-2 expression on a variety of human tumor cell lines relative to nonexpressing cells (data not shown). DC incubated with control cell debris showed slightly enhanced MLR stimulatory capacity (Fig. 7A). In contrast, exposure of DC to cell debris containing mEGP resulted in a dose-dependent diminution of stimulatory capacity. The same effect was observed when tumor debris containing mEGP was used to pulse DC in the presence of the OVA peptide Ag and D011.10 T cells (Fig. 7B). We conclude that mEGP can exert its inhibitory effect on T cell stimulation even if provided exogenously to the DC. We postulate that a similar process may occur in vivo when the DC encounters tumor Ags in the context of mEGP/GA733-2 expression by the tumor.

The immune response to adenoviral Ags is inhibited following in vivo exposure to adenovirus and mEGP

To determine whether mEGP expressing DC are functional in vivo, we examined the immune response to adenoviral Ags following i.v. vaccination. Untransduced DC or DC transduced with Ad.mEGP or control Ad.Bgl were injected into the tail vein of adenovirus naive mice. Seven days later, splenocytes were isolated and allowed to proliferate in coculture with autologous DC transduced with Ad.Bgl as a measure of adenovirus-specific T cell responses. As expected, splenocytes from mice immunized with untransduced DC showed no in vitro proliferative response to Ad.Bgl-transduced DC, whereas those from mice immunized with Ad.Bgl-transduced DC showed significant proliferation (Fig. 8A). Relative to the latter, splenocytes from mice immunized with Ad.mEGP-transduced DC had a significantly diminished T cell proliferative response to adenoviral Ags (Fig. 8A). Concomitantly, sera from mice immunized with Ad.mEGP-transduced DCs had significantly lower titers of adenovirus-specific Abs than sera from mice immunized with Ad.Bgl-transduced DCs (Fig. 8B); this reduction was evident among all Ab isotypes tested. This demonstrates that mEGP can exert its inhibitory effects when T cell stimulation takes place in vivo.

Discussion

The natural immune response to most tumors is either absent or very inefficient due to several mechanisms that allow tumor cells to regularly escape immune surveillance. T cell activity can be directly inhibited by immunosuppressive factors, such as IL-10, that are secreted either by the tumor (43, 44) or by macrophages following stimulation with the tumor (45). Many tumors down-regulate gene expression of particular MHC class I loci, β2-microglobulin (reviewed in Refs. 46 and 47), or molecules required for MHC class I-associated Ag processing such as transporter associated with Ag processing (TAP)1/TAP2 (48) as a means to evade cytotoxic CD8+ T cell responses. Similarly, several viruses have evolved elaborate mechanisms by which MHC class I molecule expression and Ag processing are blocked in infected cells (49, 50). In contrast to these well-characterized mechanisms to evade Ag presentation to CD8+ T cells, there exist few examples by which tumor cells or viruses interfere with MHC class II Ag presentation. Tumor infiltrating DC in human breast cancer (51) and in a rat colon cancer model (52) have been shown to be inefficient in Ag presentation; this effect may be due in part to the suppressive effects of IL-10 on MHC class II expression and Ag processing (53), but other more direct mechanisms, like those described for Ag processing by MHC class I molecules, could potentially exist. For example, the human CMV protein US2 induces the degradation of HLA-DRα and -DMα (54), thus preventing

![Table 1. MHC class II, but not class I, mediated T cell activation is inhibited by mEGP](http://www.jimmunol.org/Downloadedfrom/fig6.png)

<table>
<thead>
<tr>
<th>T Cell Hybridoma</th>
<th>DC: No Virus</th>
<th>DC: Ad.Bgl</th>
<th>DC: Ad.mEGP</th>
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<td>MHC class I</td>
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<td>RF3370 (100%)</td>
<td>518 ± 23</td>
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<tr>
<td>MF22D9 (100%)</td>
<td>13,200 ± 2,320</td>
<td>12,700 ± 1,030</td>
<td>4,650 ± 1,820</td>
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</table>

* DC were pulse with OVA protein and cocultured with either RF3370 or MF22D9 hybridoma cells. IL-2 secretion was assessed by ELISA as a measure of Ag T cell activation. Results are normalized to untransfected DC (percent of control).

![FIGURE 6. GA733-2 expression inhibits Ag presentation by human DCs. DCs derived from peripheral blood of healthy human donors were untransduced (no virus) or transduced with Ad.GA733 or Ad.Bgl as a control. A. Varying numbers of DCs were used as stimulators in an allo- geneic MLR using 105 CD4+ T cells from different healthy donors. B. Varying numbers of DCs were used as stimulators in an allogeneic MLR using 105 CD4+ T cells from the same donor as a measure of the proliferative response to adenovirus Ags. C. DCs (105) were mixed with 105 CD4+ T cells from the same donor and the indicated concentration of SEB; T cells were incubated alone with SEB as a control (no DC). As with the MLRs, proliferation was measured by [3H]thymidine uptake after 6 days of culture.](http://www.jimmunol.org/Downloadedfrom/fig6.png)
MHC class II-dependent Ag presentation and consequent activation of virus-specific CD4^+ T cells. We believe that this is the first study to report comparable tumor-mediated effects. We show that mEGP and GA733-2, tumor-associated glycoproteins of unresolved function, possess an activity that down-regulates MHC class II-dependent Ag presentation by bone marrow-derived DC. mEGP-mediated inhibition of DC-induced T cell activation was overcome by coculture with exogenous TCR stimuli, such as Con A, anti-CD3 Ab, or SEB (Fig. 2). Moreover, exposure of T cells to mEGP-expressing DC did not diminish the response to mEGP-nonexpressing DC as above were used to stimulate DO11.10 T cells, and proliferation was measured 3 days later. Exposure to cell debris containing mEGP inhibited Ag-specific T cell proliferation.

MHC class II-associated Ag presentation by DC was inhibited not only by expression of endogenously synthesized mEGP, but also after mEGP was internalized following exposure to cell debris from mEGP-expressing cells (Fig. 7). In both cases, the level of mEGP expression over background driven by recombinant adenoviruses was comparable to the level of endogenous GA733-2 expression on human tumors relative to background, suggesting that the effects of mEGP expression were physiologically relevant. These data also suggest that contact of T cells with mEGP alone is insufficient to mediate the inhibitory activity. This interpretation is further supported by the failure of the recombinant mEGP extracellular domain, purified from insect cells in which it was expressed using a recombinant baculovirus system, to affect T cell proliferation by mEGP-nonexpressing DC (data not shown). Taken together, our observations indicate that mEGP expression blocks Ag presentation by DCs.

The inhibitory activity of mEGP was observed both in Ag-specific, class II-restricted models of Ag presentation and in MLR, which is based on the recognition of a broad array of peptides bound to allogeneic MHC class II molecules. These observations lead us to conclude that mEGP blocks Ag presentation by DC in an Ag-independent manner, and thus likely affects presentation by most or all MHC class II molecules. Surprisingly, expression of mEGP did not alter the recognition of DC peptide/MHC class I complexes by T cells, indicating that the inhibitory effect was limited to Ag presentation by MHC class II molecules. Moreover, the effect of mEGP on the T cell proliferative response can be seen following in vivo vaccination with DC carrying both mEGP and adenoviral Ags (Fig. 8). Thus, our data provide the first published evidence of a TAA interfering with MHC class II-dependent Ag presentation by APC. Whether the effects are specific to the MHC class II/peptide-TCR interaction or to other cell surface interactions between DCs and CD4^+ T cells remains to be determined.

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Moreover, the comparison to control adenovirus-transduced controls rules out any nonspecific effects of adenovirus infection. DC are known to effectively internalize cellular fragments from apoptotic or necrotic cells and process these proteins for presentation by MHC class I and class II molecules (25, 35). Should tumor infiltrating DC internalize such fragments from mEGP-expressing tumor cells in vivo, DC presentation of TAA and infectious expression of mEGP will likely provide insights into mechanisms of are somehow mediated by the interaction of mEGP with the actin assays (data not shown). Indirect effects of mEGP on MHC class mEGP does not inhibit DC cathepsin L or S activity in in vitro further evidence against a direct interaction in vivo. Additionally, could potentially disable an essential component in the establish-ment and maintenance of an anti-tumor immune response (4) and permit escape from immune surveillance. Our data may potentially explain why women with GA733-2-expressing breast cancers have a poorer prognosis than women with tumors that lack GA733-2 expression (55).

How might mEGP inhibit Ag presentation by DCs? Some bacterial infections interfere with intracellular trafficking, and hence peptide loading, of MHC class II molecules (56, 57). These effects often correlate with a decrease in cell surface MHC class II mole-cules, comparable to the decrease in MHC class I surface expres-sion upon inhibition of MHC class I complex assembly induced by viral agents (50) or by loss of expression of TAP transporters in tumor cells (48). We were thus surprised that we could not detect any reproducible decrease in cell surface expression of MHC mole-cules or of any costimulatory molecules (Fig. 1) in DC expressing mEGP. mEGP expression did result in some defects in MHC class II complex assembly, but these did not affect the steady state levels of MHC class II/peptide complexes (our unpublished results) and were not sufficient to account for the dramatic loss of Ag presen-tation observed in mEGP-expressing DCs. The mechanism by which mEGP effects this inhibition of DC function is not yet clear. One potential mechanism would be by a direct interaction between mEGP and either MHC class II components or invariant chain (Ii). Such an interaction might be predicted to be mediated by oli-gomerization of thryoglobulin-like domains present in both mEGP (19) and the p41 form of Ii (58–60), which could recruit Ii from assembling class II complexes. Thus far, we have not been able to demonstrate such interactions in detergent extracts of mEGP-expressing DCs, and we observe no cocapping of mEGP and MHC class II molecules on the surface of DC (not shown), providing further evidence against a direct interaction in vivo. Additionally, in contrast to p41 for which the thryoglobulin-like domain inhibits cathepsin L activity in vitro and in vivo (61–63), expression of mEGP does not inhibit DC cathepsin L or S activity in in vitro assays (data not shown). Indirect effects of mEGP on MHC class II complex function or APC/T cell interactions are thus more likely to explain the inhibition of Ag presentation. Perhaps these effects are somehow mediated by the interaction of mEGP with the actin cytoskeleton (24, 64). Further analyses of DC function upon ex-pression of mEGP will likely provide insights into mechanisms of control of DC Ag presentation function by TAA and infectious agents.

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References


