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TITLE: The Role of Breast Cancer Derived Prostaglandin E2 in the Elaboration of a Therapeutic Immune Response

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The principal goal was to understand why breast cancer cells are able to evade the host immune system despite the presence of tumor antigens and tumor antigen-specific T lymphocytes. We had previously demonstrated that tumor-derived prostaglandin E2 (PGE2) directly contributes to the lack of a significant immune response to breast cancer cells. However, the production of PGE2 by breast cancer cells did not completely explain the immune suppressive effect of breast cancer cells. We have subsequently demonstrated that GA733-2/mEGP, a type I cell surface breast cancer protein, is able to efficiently block the presentation of a variety of antigens from dendritic cells (DC). Murine DC expressing mEGP were unable to stimulate allogeneic T cell responses or responses to model tumor antigens. T cell inhibition is the result of a direct effect on the DC. When mEGP or GA733-2 was provided to the T cell stimulation assay in the form of tumor debris, T cell activation was also inhibited. We postulate that mEGP blocks antigen loading into MHC complexes are further pursing the mechanism of action.
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Human breast cancer derived PGE$_2$ inhibits B7-1 induced T cell proliferation

Key words: breast cancer, immunotherapy, immunosuppressive factors, GA733-2, mEGP, antigen presentation

INTRODUCTION

The principal goal of this study is to understand why breast cancer cells are able to evade the host immune system despite the presence of tumor antigens and tumor antigen-specific T lymphocytes. We postulated that the production of prostanoids, principally prostaglandin E$_2$ (PGE$_2$), by the tumor directly contributes to the lack of an immune response to breast cancer cells. As reported year we conducted experiments to show that human breast cancer cells secrete soluble agents that directly inhibit T lymphocytes. We demonstrated that one of the major inhibitory factors made by breast cancer cells is PGE$_2$. This demonstrates that an important function of PGE$_2$ is to directly alter or suppress the immune response to breast cancer cells. We showed that the expression of cyclooxygenase (COX) and the resultant production of PGE$_2$ are sufficient to abrogate the T cell response to tumor cells in a vaccination model. The initially proposed first year Aims and related Tasks were largely completed as described in last year’s report. In brief, we reported on breast cancer cells inability to stimulate T cell proliferation due to the release of immunosuppressive factors by these tumor cells. In support of this hypothesis we observed that conditioned media (CM) obtained from breast cancer cell lines inhibited the proliferation of mononuclear cells. PGE$_2$ was shown to be an important contributor to the T cell inhibitory effect of breast cancer CM. Several lines of evidence supported this conclusion. Indomethacin treatment of MCF-7 cells reduced PGE$_2$ production and partially alleviated inhibition of MN cell proliferation. Indomethacin did not completely remove the inhibitory effect, which is consistent with the presence of residual PGE$_2$ that could be detected by LC-MS. More conclusive evidence for the involvement of PGE$_2$ in T cell growth inhibition was obtained when we removed PGE$_2$ from CM using an affinity column that specifically binds PGE$_2$. The selective elimination of PGE$_2$ from MCF-7 CM removed its MN cell growth inhibitory activity completely. Thus, inhibition of MN cell proliferation is mediated, at least in large part, by PGE$_2$ produced by MCF-7 cells. We also noted that the amount of PGE$_2$ production did not linearly correlate with inhibition of the proliferation of stimulated MN cells. CM from the immortalized, non-tumorigenic human breast epithelial cell line HBL-100 produced significant levels of PGE$_2$ (more than MCF-7) but did not suppress MN cell proliferation. Similarly, the CM from two cell lines (SUM149PT and SUM190PT) that produced the most PGE$_2$ showed only moderate inhibition of MN cell proliferation. Based on these results it appeared likely that other breast cancer factors likely contributed to the immunosuppressive effect. In support of this notion we found that BT-20 cells did not produce significant amounts of PGE$_2$ but inhibited PHA-dependent proliferation of MN cells by 41%. This suggests that other tumor-derived factors may induce immunosuppression as previously reported. Taken together these data suggest that PGE$_2$ is a necessary but not always sufficient cofactor of tumor-derived immunosuppression and likely act in concert with other factors. These data suggested that PGE$_2$ derived from human breast cancer cells can contribute to inhibition of cellular immunity but it remained unclear how to mediate reversal of tumor-induced immunosuppression as part of cancer therapy given the complexity and heterogeneity of tumor mediated immune suppression.
BODY OF REPORT

At this point in our investigations we had proposed to pursue Specific Aim 2. We proposed to determine whether inhibition of prostaglandin synthesis in vivo enhances the immunogenicity of murine mammary tumors in vivo. We proposed to use homologous recombination methods to prepare COX knockout cell lines from SCK, T2994 and/or MT901 cells using methods that had been previous described for the COX-knockout mice. However, two major obstacles prevented the pursuit of this objective. First, it became apparent that somatic cell knockout cell lines could not be prepared as proposed due to the aneuploid and unstable karyotype of the cell lines that we had available for this purpose. This was in fact alluded to by our reviewers at the time of the proposal submission. Secondly, even if COX null cell lines were prepared it would be difficult to assess the relative contribution of PGE2 to immunosuppression given our previous observations that PGE$_2$ was but one of several factors that contribute to immune suppression. The single-minded pursuit of PGE$_2$ as a therapeutically targetable factor in breast cancer progression seemed unsupportable. Therefore, we altered our approach and decided to improve our understanding of other factors that, along with PGE$_2$, contributed to immune suppression. Thus, the proposed tasks 8 through 11 were not pursed since the represented unachievable goals based on the best available information. We have gone on to characterize the previously unreported immunosuppressive effects of a well-known tumor antigen GA733-2 (also known as mEGP in the mouse).

GA733-2 is a type I transmembrane protein glycoprotein protein that is expressed on breast cancer cells and on some normal tissues [1]. The role of this protein is not well understood, although it has been postulated to be an adhesion molecule [2]. Our preliminary work has focused on the murine homologue of GA733-2 called mouse epithelial glycoprotein (mEGP). The mEGP protein has 82% sequence homology to GA733-2 and a similar tissue distribution [3, 4]. We believe that our preliminary data in the murine system provides direct and compelling evidence to support the study of the human protein in in vitro models. We have found that the mEGP is expressed on spontaneously occurring mouse mammary tumor cells such as the SCK cell line which arose naturally from an A/J mouse [5], and the NT5 cell line which was derived from spontaneous mammary tumors arising in the HER2/neu FVB transgenic mouse. In contrast, chemically induced murine mammary tumor cell lines do not (e.g., MT901 & T2994 lines; our unpublished data). GA733-2 has been shown to be expressed on human breast cancer cell lines although the role of GA733-2 in breast cancer is unknown [1, 6, 7]. The similarity of the mEGP and GA733-2 sequences and the expression of GA733-2 and mEGP on human breast and mouse mammary tumors, respectively, strongly suggest that the biologic behavior of the murine protein will predict the behavior of the human protein.
mEGP Blocks Class II Restricted Antigen Presentation in Murine Dendritic Cells. In a series of in vitro experiments we have established that mEGP: (1) blocks antigen presentation in mixed lymphocyte reaction (MLR) and MHC class II restricted antigen-specific T cells activation; (2) inhibits T cell activation by direct action on the antigen presenting cell (APC) and not by directly inhibiting the T cells; and (3) can inhibit dendritic cells (DC) either when expressed in the DC (from a transfected gene) or when the DC takes up the mEGP protein from the external environment. These data strongly suggest that one function of the mEGP and GA733-2 molecules are to block MHC class II presentation of tumor-derived antigens as shown in figure 1.

Figure 1. Endogenously synthesized (adenoviral vector delivered) mEGP or GA733-2 (G) traffics to the endosome from the endoplasmic reticulum. Endocytosed mEGP or GA733-2 (G) is taken up by the same pathway tumor antigens enter the DC. We hypothesize that in the endosome mEGP/GA733-2 is able to block formation of the peptide-MHC (class II) complex and thereby block the MLR. By this mechanism, exogenous GA733-2 from breast cancer cells would be taken up with tumor antigens and block their presentation to CD4+ T cells.
mEGP blocks antigen presentation in mixed lymphocyte reaction (MLR) and in antigen specific T cells activation. Our central model has been the MLR, where allogeneic T cells are activated by bone marrow-derived dendritic cells (BMDC). When BMDC from BALB/c mice are mixed with C3H derived purified T cells, T cell proliferation (as measured by $^3$H-thymidine incorporation) increases as a function of the number of murine BMDC (fixed number of T cells, see figure 2). The level of $^3$H-thymidine incorporation is unaltered by transfection of the BMDC with a control adenovirus (Ad.Bgl2, no transgene) or with an adenovirus expressing GA733-2 (Ad.GA733). However, transfection of the murine BMDC with an adenovirus expressing mEGP (Ad.mEGP) completely blocks the MLR response (figure 2, and figure 1, endogenous pathway of mEGP/GA733-2 entry into endosome). This is also seen when other MHC-disparate combinations of murine BMDC and T cells were used (not shown). In addition, the control culture and those containing BMDC transfected with Ad.GA733 or Ad.Bgl2 produce identical amounts of IL-2, IFNγ, IL-10 and IL-4, whereas the MLR contain the mEGP expressing BMDC fail to induce production of these cytokines (not shown). These data suggest that T cell activation fails to occur when mEGP is expressed in the BMDC. Both the BMDC and the T cells remain viable in the mEGP-containing MLR as evidence by: (1) the microscopic appearance of the cells in culture (with and without trypan blue); (2) the production of IL-12 (BMDC derived) was the same in all of the MLRs including the mEGP-containing cultures; and (3) the ability of added anti-CD3 or ConA to induce T cell proliferation when added to the mEGP-containing MLR (not shown). Thus, the absence of T cell proliferation and cytokine production in the present of mEGP is not due to cell death or other non-specific causes. These and additional data suggest that mEGP inhibits T cell proliferation by direct action on the antigen presenting cell (APC) and not by directly inhibiting the T cells. To confirm these findings in different model systems, we used T cells from transgenic mice expressing class II restricted T cell receptors (TCR) specific for either ovalbumin (OVA) or hen egg lysozyme (HEL) antigens. Using the same experimental design as employed for the MLR above, we found that BMDC pulsed with OVA or HEL proteins were unable to initiate antigen specific T cell activation of their respective transgenic T cells when mEGP (but not controls) was expressed in the stimulating dendritic cells (not shown). Both the OVA and HEL models are models of class II restricted antigen presentation. These data substantiate the ability of mEGP to block T cell responses to relevant antigens. We have also found (not shown) that murine class II restricted T cell hybridomas specific for OVA failed to respond to DC expressing mEGP, whereas murine class I restricted T cell hybridomas specific for OVA did respond to DC expressing mEGP (hybridomas provided by Dr. Kenneth Rock). This confirms the class II restricted effects of mEGP inhibition of the MLR.

mEGP can inhibit T cell activation when the APC takes up the mEGP protein from the external environment. To determine whether tumor cell-derived mEGP (or GA733-2) could block APC function, we repeated the MLR experiment and added tumor cell debris containing mEGP (exogenous pathway of mEGP/GA733-2 entry into endosome, figure 1). FBL cells (mEGP and GA733-2 negative, data not shown) were transfected with control adenovirus (Ad.Bgl2) or the adenovirus expressing mEGP (Ad.mEGP). The FBL cells were pelleted from their tissue culture media then lysed by repetitive freeze thawing. As shown in figure 3, BALB/c BMDC induce proliferation of allogeneic (C3H) T cells as shown in figure 3. When increasing amounts of mEGP$^+$-FBL3 cell debris (as measured by protein concentration) was added to the MLR, T cell proliferation was suppressed. This was not seen in the controls where increasing amounts of mEGP$^-$-FBL3 cell debris was added. These data suggest that exogenous mEGP protein can block the MLR in the same manner as endogenous mEGP (produced by adenovirus transfection of BMDC). The same effect has been seen when purified recombinant mEGP
protein (produced in bacteria as a GST fusion protein) was added to the MLR (not shown). Both endogenously and exogenously produced mEGP converge in the endosome: the site of MHC class II loading (figure 1). Therefore, we hypothesize that mEGP/GA733-2 inhibits the MLR by acting in the endosomal compartment. These data demonstrate that tumor cell derived mEGP can block T cell activation and suggests that mEGP and its human homolog GA733-2, can serve to block T cell activation in the tumor microenvironment. Our data suggest that the breast tumor infiltrating DC may be inactivated, with respect to class II antigen presentation, following encounter with the tumor cell debris (e.g., apoptotic bodies). APC (DC) within the tumor would be expected to engulf tumor debris including tumor-associated antigens for processing. However, in the process of taking up tumor-associated antigens, the APC would be expected to also take up mEGP or GA733-2, which is highly abundant. In doing so, we postulate that the DCs can no longer effectively present the tumor antigens. We are currently pursuing the mechanism by which mEGP blocks antigen presentation in our mEGP/mouse model systems. The murine system is well suited for such mechanistic studies because of the availability of unique immunologic reagents. However, at this juncture, it is critical to establish that the human protein has a similar function in patients with breast cancer. We postulate that GA733-2 contributes to the failure of the immune system to recognize breast tumor antigens, and potentially to the overall immune suppression seen in patients with advanced metastatic breast cancer.

**Human GA733-2 blocks a human MLR.** We performed a single preliminary experiment where adenovirus expressing GA733-2 was used to transduced human PBMCs for use as APCs. Using unactivated PBMCs (no IL-4, GM-CSF, etc.) GA733-2-PBMCs failed to elicit a MLR under conditions where Ad.Bgl2 transduced PBMC and control APCs did elicit an MLR response (Figure 4). The mEGP-MLR was suppressed to approximately 50% the extent of the GA733-2-MLR suggesting that while GA733-2 is inactive in the mouse model, the murine protein may have some activity in the human model. However, the use of PBMCs in this manner is difficult since they require very high amounts of adenovirus (MOI of 10,000) as they are not easily or completely transduced by adenovirus vectors. For this reason we propose to use in this proposal PBMC derived DC as APCs which are easily transduced by adenoviral vectors.

**Homology between mEGP and Invariant chain (Ii).** In the course of these studies we sought to identify homology between mEGP and other proteins that might shed light on its function. mEGP contains a thyroglobulin domain, which is a structural element first found in thyrogbulin and characterized by the sequence motif Cys-Trp-Cys-Val [8]. Similar domains have been described in other proteins including the p41 form of invariant chain [9] and equistatin (a protein derived from sea anemone, [10, 11]). In p41 invariant chain and in equistatin it has been shown that the thyroglobulin domain acts as an inhibitor of cysteine proteases. p41 invariant chain inhibits cathepsin L, but not cathepsin D [12-15], equistatin inhibits cathepsin D, B, and L as well as papain [11]. The example of equistatin, which contains three thyroglobulin domains, illustrates that subtle differences in thyroglobulin domains have significant implications for their function. Although the three thyroglobulin domains of equistatin have similar amino acid sequences, they inhibit different proteinases [11]. The presence of a thyroglobulin domain in mEGP suggested that mEGP might play a role in regulating cathepsin activity. In the context of antigen presentation, the cathepsin proteases in the endosomal and lysosomal compartment are known to play a critical role [16]. For example, cathepsin L and S are necessary for the late stages of invariant chain degradation in the thymic cortex [17], and in the thymic medulla and in peripheral APC, respectively [18]. Cathepsins are also thought to mediate antigen
processing into peptides, and a balance of protease activity seems to be necessary for efficient antigen presentation to fully degrade invariant chain but to prevent enzymatic destruction of antigen determinants [15]. It has been shown that p41 invariant chain, which blocks cathepsin L function, can increase MHC class II antigen presentation in a subset of antigens [19]. By comparison, if cathepsin S is blocked in APCs by an inhibitor or is absent (e.g., in knockout mice), the ability of MHC class II complexes to load and present antigen is impaired [20-22]. To assess the effect of mEGP on cathepsin activity in dendritic cells, we used a fluorometric assay [15]. The metabolism of the substrate used was increased in the mEGP transfected cells (Figure 5). The specificity of this increase is demonstrated by the ability of a known cathepsin-specific inhibitor to reverse block the rise in cathepsin activity. Thus, mEGP appears to activate cathepsins. This could occur by either direct interaction of mEGP with cathepsin or indirectly by blocking a negative regulator of cathepsin activity (e.g., a p41 fragment). Since cathepsins are crucial for the degradation of Ii and blockage of cathepsin S results in loss of Ag presentation in dendritic cells, we went on to perform pulse chase experiments to analyze the effect of mEGP on invariant chain and MHC class II proteins.
Figure 2. (above) Mouse MLR. BMDC expressing mEGP (◇) fail to elicit T cell proliferation, under conditions where BMDC expressing GA733-2 (○) or control BMDC (□) elicit T cell proliferation. NIH3T3 fibroblasts (□) serve as a negative control. Ad.Bgl2 transfected BMDC (△, only adenoviral proteins made) serves as an additional control.

Figure 3. Addition of tumor cell debris containing mEGP protein to BMDCs blocks MLR. FBL cells (mEGP and GA733 negative) were transfected with control adenovirus (Ad.Bgl2) or the adenovirus expressing mEGP (Ad.mEGP). The FBL cells were pelleted from their tissue culture media then lysed by repetitive freeze thawing. BALB/c untreated BMDC (□) induce proliferation of allogeneic (C3H) T cells (compare with figure 2). When increasing amounts of mEGP*-FBL cell debris (as measured by protein concentration) was added to the MLR, T cell proliferation was suppressed (hatched □, ◇ & ○). This was not seen in the controls where increasing amounts of mEGP*-FBL cell debris was added (open □, ◇ & ○). These data suggest that exogenous mEGP protein can block the MLR in the same manner as endogenous mEGP (produced by adenovirus transfection of BMDC).
**Figure 4.** Human PBMCs as APC with allogeneic T cells in a human MLR. PBMCs with or without Ad.Bgl2 induce T cell proliferation (\(\diamond, \square\) respectively). PBMCs transduced with Ad.GA733 or Ad.mEGP inhibit the MLR when approximately 40% of the cells express GA733 or mEGP respectively.

**Figure 5.** The effect of mEGP on Cathepsin Activity in DC. Increasing numbers of dendritic cells \(\left(10^4 \text{ to } 10^5\right)\) were incubated with 120uM of the cathepsin-specific fluorogenic substrate 7-amino-4-methylcoumarin-carbobenzoxy-Phe-Arg (Peninsula Laboratories, Belmont, CA) in the absence or presence of 25uM Z-Phe-Ala-CH\(_2\)F, a cysteine protease inhibitor (Enzyme Systems, Livermore, CA). Assays were conducted for 60 minutes at 37°C in 100ul PBS. Enzyme activity was determined by quantitating the fluorescence released on hydrolysis of the substrates using a multi-well plate reader (Rainbow, Austria) adjusted at an excitation wavelength of 360nm and an emission wavelength of
460nm. Enzymatic activity is directly proportional to the number of DC (amount of enzyme), completely inhibited by a specific inhibitor, and increased in the presence of mEGP

**KEY RESEARCH ACCOMPLISHMENTS**

These studies demonstrate that:

1. mEGP when ectopically expressed in BMDC blocks an allogeneic “mixed lymphocyte reaction” (MLR) as assessed by T cell proliferation, IL-2 production and interferon-γ production.
2. Inhibition of T cell activation by mEGP is dose dependent, and exhibits no “trans” effect.
3. T cell activation in the MLR is restored in the presence of mEGP when Con A or anti-CD3 antibody is added to the MLR, however, antibodies to mEGP do not restore T cell responses.
4. mEGP blocks the response of lymphocytes with transgenic T cell receptors for OVA and HEL both when the intact protein is used as the antigen or when the specific class II restricted peptide is used as the antigen.
5. mEGP is able to block class II but not class I restricted presentation of OVA antigen.
6. mEGP when provided in the form of a lysed tumor cell expressing mEGP is also able to block T cell activation as assessed in both the MLR and OVA model experiments.
7. A truncated form of mEGP lacking the cytoplasmic domain is not able to block T cell activation when expressed in the BMDC but is able to block T cell activation when provided in tumor cell debris. This also holds true for the human antigen GA733.
8. mEGP does not alter BMDC morphology, cell surface expression of key T cell stimulatory molecules (e.g., B7-1, B7-2, class I, class II, CD 11b, CD 11c), production of IL-12 and overall viability.

**REPORTABLE OUTCOMES**

1. A manuscript is being prepared for submission to Nature Medicine.
2. Our finding that proteins in the membrane of breast tumor can inhibit an immune response is the basis of a RO1 grant application to further pursue this finding. “Inhibition of T cells by a Breast Tumor Assoc. Antigen”, NCI, reviewed in 1999 with a percentile ranking of 7%. A notice of award has not as yet been received pending congressional approval of the NIH budget.

**CONCLUSIONS**

We have provided evidence that tumor-derived PGE₂ limits the immune response to breast cancer cells in an experimental model. In addition, certain membrane proteins in breast cancer cells (GA733-2 antigen) appear to block T cell responses by indirectly interfering with antigen presentation by professional antigen presenting cells.
REFERENCES


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1995-1999 Selected Topics in Chemistry (Chemistry 700)
1996-1999 The Molecular Basis of Gene Therapy, (CAMB 610)
1994-2000 Medicine 101C, Differential Diagnosis
1997-1999 Introduction to Gene Therapy (CAMB 610, Fall)
1999, 2000 Advanced Seminar in Cancer Gene Therapy (CAMB 633, Spring 1999) Course Director
1997 Wistar Cancer Biology Graduate Student Seminar
1997, 1998 Cancer Biology and Genetics Course (Pathology, Fall)
1998, 1999 Topics in Cancer Pharmacology (PHARM, Fall 1998, 1999)
1999, 2000 Cancer Pharmacology 560
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June 14, 1995  "Replication Competent Adenovirus Safety Issues", Food and Drug Administration, International Conference on Viral Safety and Evaluation of Viral Clearance from Biopharmaceuticals Products, Bethesda, MD.


October 1, 1995  "Treatment of Primary CNS Tumors with Adenovirus Mediated Gene Transfer," The GAAC Meeting on Gene Therapy, Seone, Germany.

January 18, 1996  "Adenoviral-Mediated Therapy of Brain Tumors", The Preuss Foundation Meeting on Gene Therapy for CNS Malignancies, Salk Institute, La Jolla, CA.

February 1, 1996  "Cancer Gene Therapy", Cooper Medical Center, Department of Medicine Grand Rounds, Camden, NJ.

April 17, 1996  "Gene Therapy", The Estelle Lasko Memorial Lecture, The Twenty-fourth Annual Chester County Cancer Conference, Chester County Hospital, West Chester, PA.

April 19, 1996  "Gene Therapy for Inherited and Acquired Diseases", Genetics in the Cause and Treatment of Malignancies Conference, Sacred Heart Hospital, Allentown, PA.

May 13-7, 1996  "Laboratory and Clinical Approaches to Cancer Gene Therapy" 1996 Short Course in Cancer Biology, University of Nebraska Medical Center, Omaha, NE.


July 11, 1996  "Cancer Gene Therapy" Shering-Plough Corporation, Kenilworth, NJ.


Jan. 9, 1997  "Adenoviral Vectors for the Gene Therapy of Cancer", Wayne State University, Center for Molecular Medicine and Genetics, Detroit, MI.

March 7, 1997  Gene Therapy for Gliomas and Colon Cancer, University of South Carolina, Department of Microbiology, Charleston, SC.

April 25, 1997  "Colon Cancer Vaccines", Megabios Corporation, Burlingame, CA.


Aug. 7, 1997  "Gene Therapy of Malignant Gliomas" Cancer Section, Gorden Conference, Newport, RI.


Oct. 20, 1997  "Experimental Therapies for Malignant Gliomas", Pathology Grand Rounds, Suburban General Hospital, Norristown, PA.


March 28, 1998  "Phase I Trial of Gene Therapy for Primary Brain Tumors", Cerebral Vascular Biology 1998 Conference, Portland (Lincoln), OR.

June 29, 1998  "Phase I Trial of Gene Therapy in Primary Brain Tumors", American Society of Gene Therapy, Seattle, WA.


May 16, 1999  "Cancer Gene Therapy: Clinical Trials and Their Scientific Basis", American Society of Clinical Oncology Meeting, Atlanta GA.

June 12, 1999  "mEGP Blocks Class II Antigen Presentation" American Society of Gene Therapy, Washington D.C.

June 18, 1999  "Imaging Cancer Gene Therapy with PET" 25th Annual Pendergrast Symposium, Department of Radiology, University of Pennsylvania, Philadelphia, PA.

Sept. 29, 1999  Gene Therapy: Applications for Brain Tumors and Other Malignancies" New York Medical College, Valhalla, NY.
Oct. 19, 1999  “Mouse EGP Inhibits Antigen Presentation in Dendritic Cells: A New Molecular Pathway for Tumor-Mediated Immune Suppression”, University of North Carolina Cancer Center Grand Rounds, Chapel Hill, NC

Oct. 22, 1999  Clinical Infection Control in Gene Therapy: Clinical Applications of Gene Therapy”. University of Kentucky Medical Center, Lexington, KY.

Dec 17, 1999  “Gene Therapy for Malignant Gliomas and other Cancers, Grand Rounds, Moffitt Cancer Center, University of South Florida, Tampa, FL

January 7, 2000  Mouse Epithelial Glycoprotein: A Tumor Antigen That Inhibits Antigen Presentation” Surgery Grand Rounds, Memorial Sloan-Kettering Cancer Center, New York, NY


April 12, 2000  “Gene Therapy for Malignant Gliomas”, University of Florida Cancer Center Grand Rounds, Gainesville, FL


June 20, 2000  “New Developments in Cancer Gene Therapy”, Department of Medicine Grand Rounds, Oregon Health Sciences University, Portland, OR.

July 26, 2000  “How tumors avoid being seen: a tumor associated antigen that blocks class II mediated antigen presentation” Hematology/Oncology Division Research Conference, University of Michigan Medical Center, Ann Arbor, MI

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H. K. E. Boxhorn, M. Jost, U. Rodeck, S. Ethier, S. L. Eck. Human breast cancer cell lines inhibit the proliferation of human peripheral blood mononuclear cells by PGE2 and other immunosuppressive factors 1999 (submitted in revision)


R. Gutzmer, S. Sutterwala, E. Behrens, L. Wei, M. Marks and S. L. Eck: Mouse Epithelial Glycoprotein blocks Class II restricted Antigen Presentation in Dendritic Cells (submitted 2000).


Research Publications, non-peer reviewed


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