

Activation of Tumor-specific CD4⁺ T Lymphocytes by Major Histocompatibility Complex Class II Tumor Cell Vaccines: A Novel Cell-based Immunotherapy

Samudra K. Dissanayake,¹ James A. Thompson,¹ Jacobus J. Bosch,² Virginia K. Clements,¹ Peter W. Chen,² Bruce R. Ksander,² and Suzanne Ostrand-Rosenberg¹

¹Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, Maryland, and ²The Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts

ABSTRACT

Mouse tumor cells transfected with syngeneic MHC class II and costimulatory molecule genes are therapeutic vaccines in mice, provided they do not coexpress the class II-associated invariant chain (Ii). We demonstrated previously that the vaccine cells present tumor peptides via the endogenous antigen presentation pathway to activate CD4⁺ and CD8⁺ T cells. Because of their efficacy in mice, we are translating this vaccine strategy for clinical use. To obtain MHC class II⁺CD80⁺Ii⁻ human tumor cells, we developed retroviruses encoding HLA-DR and CD80. The HLA-DR virus encodes the DR α and DR β 0101 chains using an internal ribosomal entry site to coordinate expression. SUM159PT mammary carcinoma and Mel 202 ocular melanoma cells transduced with the retroviruses DRB1/CD80 express high levels of DRB0101 and CD80 on the cell surface in the absence of Ii. Irradiated SUM159PT/DR1/CD80 vaccines stimulate proliferation of non-HLA-DRB0101 peripheral blood mononuclear cells and present an exogenous DR1-restricted tetanus toxoid (TT) peptide, indicating that the transduced DRB0101 is functional. SUM159PT/DR1/CD80 vaccines were further transduced with a retrovirus encoding the TT fragment C gene, as a model tumor antigen. These cells stimulate IFN- γ release from TT-primed human DRB0101 peripheral blood mononuclear cells, demonstrating their ability to present “endogenous” tumor antigen. Depletion and antibody blocking experiments confirm that MHC class II-restricted, endogenously synthesized epitopes are presented to CD4⁺ T cells. Therefore, the MHC class II vaccines are efficient antigen-presenting cells that activate tumor-specific MHC class II-restricted, CD4⁺ T lymphocytes, and they are a novel and potential immunotherapeutic for metastatic cancers.

INTRODUCTION

A key goal of cancer vaccine development is to generate therapeutic reagents that provide protection against development and outgrowth of metastatic tumor cells. Because metastatic disease for many tumors appears at varied intervals after diagnosis of primary tumor, the most effective vaccines will provide long-term immune memory. We (1, 2) and others (3–5) have focused on the critical role of CD4⁺ T cells in cancer vaccines, because these cells, in conjunction with CD8⁺ T lymphocytes, are likely to provide maximal antitumor immunity with long-term immunological memory.

To better activate tumor-specific CD4⁺ T cells, we have designed cell-based vaccines that facilitate the presentation of MHC class II-restricted tumor peptides to responding CD4⁺ T cells (2). We have reasoned that tumor cells present a variety of MHC-restricted peptides that are potential tumor antigens, and that if they constitutively express MHC class I molecules and are transduced with syngeneic MHC

class II and costimulatory molecules, they could function as antigen-presenting cells (APCs) for MHC class I- and class II-restricted tumor peptides. This approach is appealing for several reasons: (a) identification of specific tumor antigen epitopes is not required; (b) multiple class I- and class II-restricted epitopes will be presented concurrently; and (c) CD4⁺ T cells may be activated to novel MHC class II-restricted tumor epitopes not presented by professional APCs.

To test our approach, cell-based vaccines were generated from three independent mouse tumors that constitutively express MHC class I molecules and do not express MHC class II molecules (mouse SaI sarcoma, B16 melanoma, and 4T1 mammary carcinoma). The mouse tumor cells were transfected with syngeneic MHC class II α - and β -chain genes and with costimulatory molecule (*CD80*) genes. This vaccine approach was adapted for two reasons:

(a) In conventional immunity, activation of CD4⁺ T cells requires the uptake of soluble antigen by professional APCs and the cross-presentation of the processed antigen to specific CD4⁺ T cells. If antigen is limiting, as it may be when tumor burden is low, available antigen may not be sufficient for the activation of tumor-specific CD4⁺ T cells. Our vaccine design bypasses the requirement for professional APCs and soluble tumor antigen because the genetically modified tumor cell vaccines function as the APC.

(b) Because each vaccine cell expresses both MHC class I and class II molecules and their associated tumor peptides, a given vaccine cell could be an APC for both MHC class I- and class II-restricted tumor antigen epitopes and concurrently activate both CD4⁺ and CD8⁺ T cells. If CD4⁺ and CD8⁺ T cells are simultaneously activated by the same APC and are in close proximity to each other, then the transfer of “help” from the activated CD4⁺ to the CD8⁺ T cell should be highly efficient (2, 6), thereby maximizing the therapeutic effect. CD4⁺ T cell “help” could be provided to CD8⁺ T cells via the classical mechanism of soluble cytokine production or by the alternative mechanism of up-regulation of CD40 on the vaccine cells (APCs; Ref. 7). Regardless of the mechanism of help, the activated CD4⁺ T cells do not need to directly interact with wild-type tumor cells or with professional APCs, because their only role is to provide help to CD8⁺ T cells.

Extensive studies using a variety of mouse tumor models have shown that immunization/immunotherapy with the MHC class II plus CD80-modified vaccines induces a potent antitumor immunity against wild-type tumor that confers prophylactic protection (1), delays or eliminates growth of primary solid tumors (8), reduces both experimental (9) and spontaneous metastasis, and extends survival (10, 11). Immunization studies using genetically marked vaccine cells have demonstrated that the vaccine cells themselves are the relevant APCs *in vivo* (12–14), and that both CD4⁺ and CD8⁺ T cells are required for the optimal antitumor effect (8, 11). Therefore, by circumventing the traditional cross-presentation pathway for activation of CD4⁺ and CD8⁺ T cells, these genetically modified cancer vaccines induce a potent tumor-specific immunity against wild-type tumor cells.

The efficacy of the vaccines depends on their ability to present endogenously synthesized, MHC class II-restricted tumor antigen epitopes to activate CD4⁺ T cells. Presentation of endogenous antigen

Received 8/22/03; revised 11/6/03; accepted 1/8/04.

Grant support: Grants R01CA84232 and R01CA52527 from the NIH and Contract DAMD-17-01-1-0312 from the United States Army Research and Development Command.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: J. A. Thompson is supported by a pre-doctoral fellowship from the United States Army Research and Development Command, Contract DAMD-17-03-1-0337.

Requests for reprints: Suzanne Ostrand-Rosenberg, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21250; Phone: (410) 455-2237; Fax: (410) 455-3875; E-mail: srosenbe@umbc.edu.

is favored when levels of the class II-associated accessory molecule, invariant chain (Ii), are limiting (reviewed in Ref. 15). Because MHC class II and Ii are coordinately regulated and Ii expression blocks vaccine efficacy (14, 16, 17), we have used tumor cells that do not constitutively express MHC class II (or Ii) as the “base” line for the vaccines.

Because of its therapeutic efficacy in mice, we are translating this vaccine approach for the treatment of human cancers. Our experimental design is to express syngeneic MHC class II and costimulatory molecules in established human tumor cell lines that constitutively express MHC class I molecules and do not constitutively express MHC class II or Ii. To achieve this goal, we are using retroviral transduction to express HLA-DR and CD80 molecules in two human tumor lines, an ocular melanoma (Mel 202) and a mammary carcinoma (SUM159PT). The resulting HLA-DRB0101-transduced cells stably express high plasma membrane levels of functional HLA-DRB0101, as measured by immunofluorescence, activation of allogeneic peripheral blood mononuclear cells (PBMCs), and presentation of a DR1-restricted peptide. To ascertain that the transductants activate CD4⁺ T lymphocytes to endogenously synthesized antigens, we have shown that tetanus toxoid (TT) fragment C-transduced vaccine cells activate TT-specific HLA-DRB0101-restricted CD4⁺ T cells. Therefore, human tumor cells genetically modified by gene transfer to express syngeneic MHC class II and costimulatory molecules express functional HLA-DR molecules and may serve as useful therapeutics for activating tumor-specific CD4⁺ T lymphocytes of cancer patients.

MATERIALS AND METHODS

Construction of Retroviral Vectors. For the pLNCX2/DR1 construct, DRA cDNA in the RSV.5 vector (18) was PCR amplified including 5' *NheI* and 3' *XhoI* restriction sites: DRA 5' primer, TGTCGCTAGCATGGCCATAAGTGGAGT; and DRA 3' primer, ACTGCTCGAGTTACAGAGGCCCTGCGTT. The PCR product was cloned into the pCR2.1-TA vector (Invitrogen, Carlsbad, CA), excised with *NheI* and *EcoRI*, and inserted into the multiple cloning site (MCS)-A of *NheI*- and *EcoRI*-digested pIRES plasmid (Clontech, Palo Alto, CA). DRB0101 in the RSV.5 vector (18) was PCR amplified including 5' *XmaI* and 3' *NotI* sites and subcloned into the 5' *XmaI* and 3' *NotI* sites of the MCS-B of the pIRES vector: DRB0101 5' primer, AGTACCCGGGATGGTGTGTCTGAAGCTC; and DRB01013' primer, TAGTGGCCGCTCAGCTCAGGAATCCTGTG. PCR conditions for both DRA and DRB0101 amplifications were: denature at 94°C for 2 min, denature at 94°C for 1 min, anneal at 60.9°C or 62.9°C (DRA and DRB0101, respectively) for 1 min, extend at 72°C for 3 min (High Fidelity Taq; Roche, Basel, Switzerland); repeat the last three steps 30 times and extend at 72°C for 7 min. The resulting construct is pIRES/DR1 (Fig. 1A).

The pLNCX2 retroviral vector (Clontech) was modified to include a linker containing an *AvrII* site in the MCS. To make the linker, equimolar amounts of the oligonucleotides (5'-GATCTCGAGCTCCTAGGAATGTTTGGCCGAGGC-3' and 3'-AGCTCGAGGATCCTTAACAAACCGGCTCCGCCGG5'-) were mixed, heated at 95°C for 5 min, and then incubated at 22°C for 1 h. The resulting linker was ligated to *BglII*- and *NotI*-digested pLNCX2. The resulting construct is pLNCX2/*AvrII*.

The DRA-IRES-DRB0101 fragment of the pIRES/DR1 was digested with *NheI* and *NotI* and gel purified using a QIAquick gel extraction kit (Qiagen, Valencia, CA) and then ligated to *AvrII*- and *NotI*-digested pLNCX2/*AvrII*. The final MHC class II construct is pLNCX2/DR1 (Fig. 1A).

For the pLHCX/CD80(HPH) construct, pLHCX (hygromycin resistance; Clontech) was modified to include a 5' *BamHI* site and a 3' *HindIII* site by inserting an oligonucleotide linker between the *HindIII* and *ClaI* sites of the MCS. The original *HindIII* in the vector was deleted by insertion of the linker. *XhoI*, *HpaI*, *AvrII*, and *NotI* restriction sites were included in the linker for future cloning purposes. The linker sequence was: L1, 5'-AGCTGCTCGAGT-TAACGGATCCTAGGAAGCTTGCGGCCGCAT-3'; and L2, 5'-CGATGCGGCCGCAAGCTTCTAGGATCCGTTAACTCGAGC-3'.

Human CD80 was excised from the pREP10/B7.1 vector with *BamHI* and

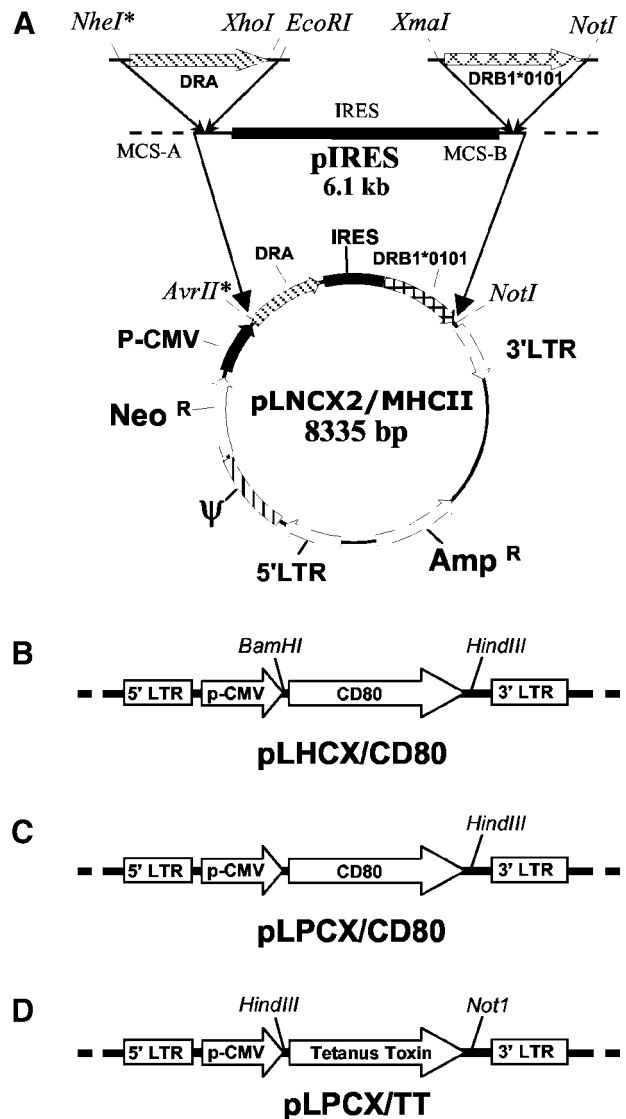


Fig. 1. Retroviral constructs made and used in these studies. A, the pLNCX2/MHC II construct contains the DRA and DRB0101 cDNAs flanking an IRES and under the control of the cytomegalovirus (CMV) promoter and contains the *G418* resistance gene. B and C, the pLHCX/CD80 and pLPCX/CD80 constructs encode the human *CD80* gene and contain the hygromycin or puromycin resistance genes, respectively. D, the pLPCX/TT construct encodes the TT fragment C gene and contains the puromycin resistance gene. LTR, long terminal repeat.

HindIII and inserted into the modified pLHCX vector using the *BamHI* and *HindIII* sites (Fig. 1B).

For the pLPCX/CD80 (Puro) construct, the *CD80* gene was excised from pREP10/B7.1 by digestion with *BglII* and *HindIII* and ligated into pLPCX digested with *BamHI* and *HindIII*. The *BamHI* and *BglII* sites were deleted during this process (Fig. 1C).

For the pLPCX/TT construct, TT fragment C DNA was PCR amplified from pCR Blunt (19) to include an ATG start codon and *HindIII* site at the 5' end and a *BamHI* site at the 3' end: 5' primer sequence, CCGCCGAGCT-TGCCACCATGAAAAACCTTGATTGTT; and 3' primer sequence, CTGT-TCGGATCCTTAGTCGTTGGTCCAA. PCR conditions were: denature at 94°C for 5 min, denature at 94°C for 1 min, anneal at 55°C for 1 min, extend at 72°C for 1 min (*Taq*DNA polymerase; Invitrogen); repeat the last three steps 35 times and extend at 72°C for 10 min. The resulting PCR product was inserted into the TA cloning vector, pGEM-T-Easy (Invitrogen). The modified TT fragment C gene was then excised with *HindIII* and *BamHI* and inserted into the mammalian expression vector pCDNA3.1/Zeo(+) (Invitrogen). A *HindIII*-*NotI* fragment containing the TT fragment C gene was then excised from pCDNA3.1/Zeo(+) and subcloned into the *HindIII*-*NotI* site of the MCS of pLPCX(Puro) to produce the pLPCX/TT vector.

Table 1 Tumor cell vaccines (transductants) used in these studies

Cell line	HLA-DRB0101	CD80	TT ^a	Drug selection
SUM/DR1	+			G418 ^b
SUM/CD80		+		HPH ^c
SUM/TT			+	Puro ^d
SUM/DR1/CD80	+	+		G418 ^b + Puro ^d
SUM/DR1/CD80/TT	+	+	+	G418 ^b + HPH ^c + Puro ^d
SUM/DR1/TT	+		+	G418 ^b + Puro ^d
SUM/CD80/TT		+	+	HPH ^c + Puro ^d
Mel 202/DR1	+			G418 ^b
Mel 202/CD80		+		HPH ^c
Mel 202/DR1/CD80	+	+		G418 ^b + Puro ^d

^a Tetanus toxin fragment C.

^b 600 µg/ml.

^c 200 µg/ml.

^d 0.2 µg/ml.

^e 75 µg/ml.

Cells. Media for all cell lines contained 1% gentamicin, 1% penicillin/streptomycin (all from BioSource, Rockville, MD), and 2 mM Glutamax (BRL/Life Sciences, Grand Island, NY). All cells and T-cell activation assays were cultured at 37°C in 5% CO₂. SUM159PT was obtained from the Michigan Breast Cell/Tissue Bank³ and was maintained in Ham's F-12 medium with 10% heat-inactivated FCS (Hyclone, Logan, UT), 1 µg/ml hydrocortisone, and 5 µg/ml insulin (both from Sigma, St. Louis, MO). Mel 202 (20) was grown in RPMI 1640 (BioSource, Rockville, MD) with 10% FCS, 0.01 M HEPES (Invitrogen, Grand Island, NY), and 5 × 10⁻⁵ M β-mercaptoethanol (J. T. Baker, Inc., Phillipsburg, NJ). Transductants were grown in the same medium as their parental cells, supplemented with G418 (Sigma), puromycin (Clontech, Palo Alto, CA), or hygromycin (Calbiochem, San Diego, CA; see Table 1 for dosages), depending on their transgenes. Sweig and Jurkat cells were obtained from the American Type Culture Collection and were maintained in Iscove's modified Dulbecco's medium (BioSource) supplemented with 10% fetal clone I (FBP; Hyclone). EBV B cells were grown in RPMI 1640 with 10% FCS and 0.01 M β-mercaptoethanol. Peripheral blood mononuclear cells (PBMCs) were grown in Iscove's modified Dulbecco's medium with 5% human AB serum (Gemini Bio-Products, Woodland, CA). All cell lines and procedures with human materials were approved by the Institutional Review Boards of the participating institutions.

Retrovirus Production. 293T cells (obtained from the Harvard Gene Therapy Institute) were plated in a 6-cm dish at 9 × 10⁵ cells/4 ml of 293T medium [DMEM (BioSource, Rockville, MD), 1% gentamicin, 1% penicillin/streptomycin, 1% Glutamax, and 10% heat-inactivated FCS] and cultured at 37°C. Twenty h later, the growth medium was replaced with 4 ml of 37°C Iscove's modified Dulbecco's medium containing 25 mM HEPES (BioSource), 1% Glutamax, and 10% heat-inactivated FCS. Three h later, the 293T cells were transfected with pLNCX2/DR0101, pLHCX/CD80, pLPCX/CD80, or pLPCX/TT plasmids (8 µg) plus pMD.MLV gag.pol (6 µg) and pMD.G (2 µg) using CaPO₄ (21). Twelve to 16 h after transfection, medium was replaced with 293T growth medium containing 10 mM HEPES. Virus was collected 48 h later and either used immediately or stored at -80°C.

Retroviral Transduction. Tumor cells were plated in 6-well plates at 1.2-3 × 10⁵ cells/3 ml growth medium/well. Approximately 16 h after plating, when cells were in log phase, growth medium was replaced with 500 µl of viral supernatant mixed with 500 µl of 293T medium containing 4 µg/ml polybrene (Sigma) and 10 mM Hepes. Cells were incubated for 5-6 hrs at 37°C, washed twice with excess PBS and maintained in growth medium for 2 days before adding G418, puromycin, and/or hygromycin.

Peptides, Antibodies, Reagents, and Immunofluorescence. TT p2 peptide TT₈₃₀₋₈₄₄ (QYIKANSKFIGITEL; Ref. 22) was synthesized at the University of Maryland Biopolymer Laboratory. Formaldehyde-inactivated TT was purchased from Accurate Chemical and Scientific Corporation (Westbury, NY).

Monoclonal antibodies [mAbs; HLA-DR-FITC, CD80-phycoerythrin (PE), and anti-TT], streptavidin-PE, FITC-isotype, and PE-isotype controls were purchased from BD PharMingen (San Diego, CA). Biotinylated HLA-DR0101 was purchased from One Lambda, Inc. (Canoga Park, CA). Rat anti-mouse IgG-FITC was purchased from ICN (Costa Mesa, CA), and CD4-FITC, CD8-FITC, and anti-human IgG-FITC were purchased from Miltenyi Biotech (Au-

burn, CA). Human IgG-FITC was purchased from Cappel (West Chester, PA). Culture supernatants of hybridomas W6/32 (pan HLA-A,B,C), L243 (pan anti-HLA-DR), 28.14.8 (anti-H-2L^d, D^b), and PIN1 (anti-Ii) were purified on protein A or protein G affinity columns as described previously (1). Tumor cells and PBMCs were stained for cell surface markers (MHC class I, class II, CD80, CD4, CD8, and immunoglobulin) or fixed and stained for internal markers (Ii, TT) by direct or indirect immunofluorescence as described previously (1, 16). PBMCs were collected by venipuncture from HLA-typed healthy donors and isolated using Histopaque 1077 separation medium as described previously (20). For some experiments, PBMCs were provided by Dr. D. Mann (University of Maryland Baltimore). PBMCs were stored at 1 × 10⁷ cells/ml at -80°C until used.

Western Blots. Western blot analyses were performed as described (14) using 10% SDS-PAGE. Blots were incubated with PIN1.1 mAb (0.003 µg/ml) followed by sheep anti-mouse HRP at a 1:10,000 dilution (Amersham).

Allogeneic T-Cell Activation. Responder PBMCs (1 × 10⁵/well) were cultured in triplicate with 5 × 10³ or 1 × 10⁴ irradiated (CS-137 irradiator; Kewaunee Scientific, Statesville, NC) stimulator SUM159PT (50 Gy) or 5 × 10⁵ allogeneic PBMCs (40 Gy) per well in 200 µl/well of culture medium (RPMI, 10% FCS, 1% penicillin/streptomycin, 2 mM Glutamax, and 0.01 M β-mercaptoethanol) in flat-bottomed 96-well microtiter plates (Corning, Inc., Corning, NY). Cells were incubated at 37°C in 5% CO₂ for 6 days and pulsed with [³H]thymidine (2 µCi/well) during the final 18 h, after which the cells were harvested onto glass fiber filter mats using a Packard Micromate 196 cell harvester (Downers Grove, IL). Filter mats were sealed into plastic bags with 5 ml of betaplate scintillation fluid (Perkin-Elmer, Gaithersburg, MD) and counted using a Wallac 1450 Microbeta liquid scintillation counter (Perkin-Elmer). Samples were run in triplicate.

Stimulation Index (SI)

$$\text{SI} = \frac{(\text{cpm of transduced experimental tumor cells} + \text{allogeneic PBMC}) - (\text{cpm of transduced tumor cells alone})}{\text{cpm of allogeneic PBMCs alone}}$$

Responder PBMCs do not express DRB0101.

TT-boosted PBMCs. DRB0101 PBMCs (2 × 10⁷ cells/4 ml/well) were cultured with 1 µg/ml of exogenous TT (Accurate, Westbury, NY) in 6-well tissue culture plates (Corning). After 5 days of culture, nonadherent cells were harvested, washed twice with Iscove's modified Dulbecco's medium, and replated in culture medium with 20 units/ml of recombinant human interleukin 2 (R&D Systems, Minneapolis, MN) at 1 × 10⁶ cells/2 ml/well in 24-well plates (Corning). Remaining nonadherent cells were harvested 7 days later, and live cells were isolated using Histopaque-1077 separation medium. TT-activated, nonadherent cells were maintained in culture medium without exogenous interleukin 2 overnight and used the following day.

Antigen Presentation Assays for Endogenous TT and Exogenous TT Peptides. Irradiated (50 Gy) stimulator cells (1 × 10⁴ or 2.5 × 10⁴ cells/well) were cocultured in triplicate with adherent cell-depleted, TT-primed DRB0101 responder PBMCs (5 × 10⁴ cells/well) in 200 µl/well in flat-bottomed 96-well microtiter plates (Corning). After 2 days of culture, supernatants were collected and assayed by ELISA for IFN-γ according to the manufacturer's instructions (Endogen, Woburn, MA). For the antibody blocking experiments, 1 × 10⁴ stimulator cells were incubated with 10 µg/ml or 12.5 pg/ml of L243 (anti-HLA-DR) or 28.14.8 (isotype-matched irrelevant mAb) in 100 µl/well for 45 min before the addition of responder PBMCs. Values are the averages of triplicate points with their SDs.

For exogenous TT peptide p2 presentation, assays were as for endogenous antigen presentation, except soluble TT peptide p2 (22, 23) was added at the beginning of the 2-day culture period, and antigen-presenting cells not transduced with TT were used.

CD4, CD8, and CD19 Cell Depletions. Adherent cell-depleted, TT-primed PBMCs were depleted for CD4⁺, CD8⁺, or CD19⁺ cells using magnetic beads, LD columns, and the QuadroMACS separation system according to the manufacturer's instructions (Miltenyi Biotech). Purity of depleted fractions was confirmed by flow cytometry.

HLA-DR Nomenclature. The PBMCs used in these studies were HLA typed by PCR; hence, they are known to be HLA-DRB0101. The HLA-DR gene used in these studies was sequenced and identified as HLA-DRB0101 and

³ Internet address: www.cancer.med.umich.edu/breast_cell/umbnkb.htm.

is abbreviated as "DR1" in the names of the transductants. The TT p2 peptide has been identified as a DR1-restricted epitope; however, its DR1 subtype is not known.

RESULTS

Construction of Retroviruses Encoding HLA-DR α Plus HLA-DR β , CD80, and TT Fragment C. To generate human tumor cells expressing high levels of MHC class II molecules, retroviruses encoding HLA-DR α plus HLA-DR β genes have been generated. The HLA-DRB0101 allele was selected because it is one of the more common alleles in the Caucasian population and is a frequently used restriction element (24, 25). A novel bicistronic retroviral vector that drives coordinate expression of approximately equimolar amounts of HLA-DR α and HLA-DR β was developed using the pLNCX2(neo) retroviral backbone. DR α and DR β 0101 cDNAs (18) were cloned upstream and downstream, respectively, of the internal ribosomal entry site (IRES) of the vector pIRES. The DRA-pIRES-DRB segment was then excised from the pIRES vector and ligated into the pLNCX plasmid to yield the pLNCX/DR β 1 plasmid (Fig. 1A). This construct will produce a single-chain mRNA driven by the cytomegalovirus promoter in which DR α is translated by a CAP-dependent mechanism and DR β is translated via the IRES in a CAP-independent manner.

Because of the critical role of costimulatory molecules in the activation of naive T cells (26), we have also generated retroviral plasmids encoding human CD80 (hCD80). The *hCD80* gene was excised from the pREP10/B7.1 plasmid and ligated into the retroviral vector pLHCX(HPH) or pLPCX(Puro) to form the pLHCX/CD80 (Fig. 1B) or pLPCX/CD80 (Fig. 1C) plasmids, respectively.

To monitor presentation of endogenously synthesized antigen, a retroviral plasmid encoding the TT fragment C was generated. The TT fragment C gene was excised from the pCR Blunt plasmid, an ATG start codon was inserted at its 5' end, and the resulting construct was ligated into the pLPCX(Puro) vector to form the pLPCX/TT retroviral plasmid (Fig. 1D). All retroviral plasmids were packaged in 293T cells, and supernatants containing infectious retroviruses were harvested and used to transduce target tumor cells.

Transduced Human Tumor Cells Express Cell Surface HLA-DRB0101 and CD80 and Internal TT. The human ocular melanoma cell line Mel 202 and the mammary carcinoma cell line SUM159PT were transduced with different combinations of the pLNCX2/DR1, pLHCX/CD80, pLPCX/CD80, and pLPCX/TT retroviruses. The resulting transductants are shown in Table 1. SUM159PT and Mel 202 tumors were chosen because they do not constitutively express MHC class II molecules and hence should not express Ii, which we have shown previously inhibits presentation of MHC class II-restricted endogenous antigens (14, 17). To assess the magnitude and stability of transgene expression, transductants were tested by immunofluorescence and flow cytometry 1 week after being placed on drug selection (see Table 1 for drug selection conditions for each transductant line) and intermittently for 6 months thereafter. As shown in Fig. 2, Mel 202 and SUM159PT transductants express high levels of cell surface HLA-DR (L243 mAb), CD80 (CD80-PE mAb), and internal TT (polyclonal anti-TT ab), as measured at 6 months after transduction. HLA-DR-expressing Mel 202 and SUM159PT cells were also biotinylated, and the cell extracts were immunoprecipitated with anti-HLA-DR mAbs to assure proper structural conformation of cell surface-expressed, transduced class II molecules. Both lines displayed high levels of SDS-stable MHC class II $\alpha\beta$ dimers, indicating proper conformation and peptide binding.⁴ The parental lines and transduc-

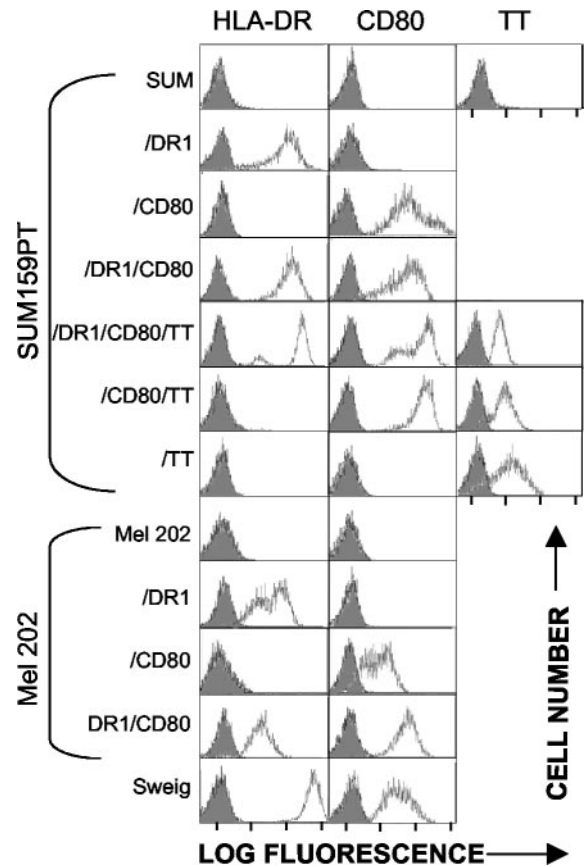


Fig. 2. SUM159PT and Mel 202 transductants express MHC class II HLA-DR and CD80 on the cell surface and TT internally. Live transductants were stained by direct immunofluorescence for plasma membrane HLA-DR (L243-FITC) or CD80 (CD80-PE). Fixed cells were stained by indirect immunofluorescence for internal TT (TT mAb plus fluorescent conjugate). Gray peaks denote staining with fluorescent conjugate alone or isotype control; white peaks represent staining with directly coupled primary antibody or primary antibody plus fluorescent conjugate. These data are from one of three to five independent experiments.

tants were also stained for MHC class I molecules (W6/32 mAb). All lines showed strong class I expression, with transductants displaying levels roughly comparable with their parental lines (data not shown).

To ascertain that the MHC class II expression is allele specific, SUM/DR1 and SUM/DR1/CD80 cells were stained for cell surface expression of HLA-DR1 using the HLA-DR1-specific mAb. As shown in Fig. 3, pLNCX2/DR1-transduced SUM cells express high levels of DR1 and only stain at background levels with an irrelevant HLA-DR2-specific mAb. Therefore, SUM/DR1/CD80, SUM/DR1, SUM/CD80, SUM/DR1/CD80/TT, Mel 202/DR1, Mel 202/CD80, and Mel 202/DR1/CD80 transductants express high levels of the transduced *HLA-DR*, *CD80*, and/or *TT* genes as measured by antibody reactivity and immunofluorescence.

SUM159PT and Mel 202 Cells Do Not Express Invariant Chain. Because coexpression of Ii inhibits endogenous antigen presentation by MHC class II vaccine cells (14, 17), SUM159PT and Mel 202 cells were tested to ascertain that they do not express Ii. Cells were permeabilized, stained with the Ii-specific mAb PIN-1, and analyzed by flow cytometry. As shown in Fig. 4A, neither tumor line contains Ii, whereas the human B cell line, Sweig, which constitutively expresses Ii, is strongly positive. To further confirm the absence of Ii, detergent extracts of SUM159PT, Mel 202, Ii-positive Sweig, and Ii-negative Jurkat cells were electrophoresed by SDS-PAGE and analyzed by Western blotting for Ii expression. As shown in Fig. 4B, neither SUM159PT, Mel 202, Mel 202/DR1/CD80, nor SUM/DR1/

⁴ V. Clements, unpublished results.

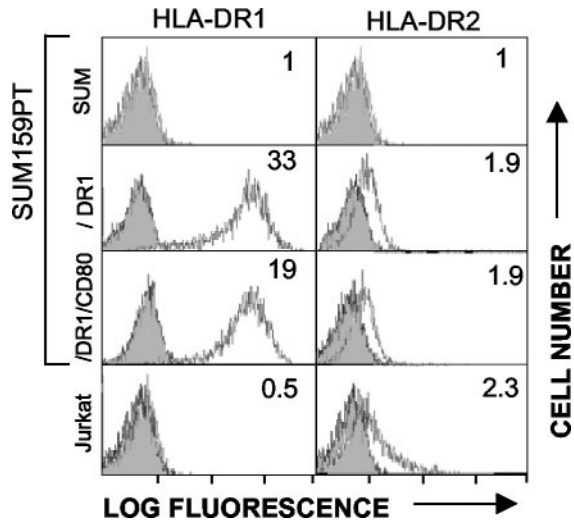


Fig. 3. SUM/DR1 and SUM/DR1/CD80 transductants express HLA-DR1 at the cell surface. Live cells were stained by indirect immunofluorescence for plasma membrane HLA-DR1 (mAb HLA-DR1 biotin) or with an irrelevant Ab (HLA-DR2-biotin) plus an avidin-PE conjugate. Jurkat is a DR1⁻ cell line. *Gray peaks* denote staining with fluorescent conjugate without primary antibody; *white peaks* represent staining with primary antibody plus fluorescent conjugate. Numbers in the upper right-hand corner of each profile are the mean channel fluorescence for the antibody stained peak. These data are from one of two to five independent experiments.

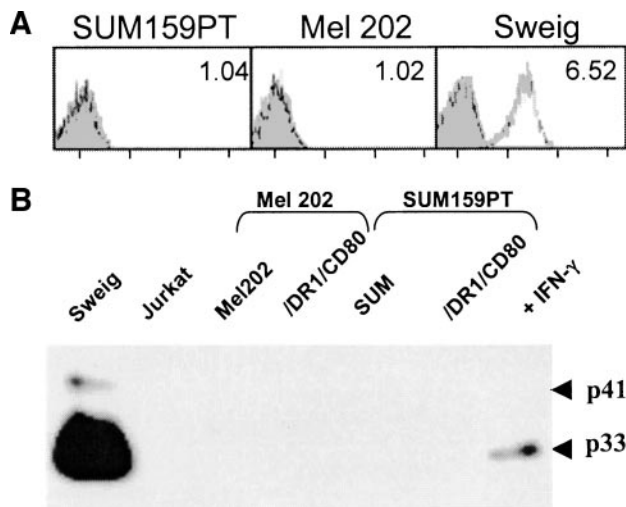


Fig. 4. Sum159PT and Mel 202 cells do not express Ii chain. *A*, fixed cells were stained by indirect immunofluorescence for Ii (mAb PIN1.1; *white peaks*) or with fluorescent conjugate alone (*gray peaks*). These data are representative of three independent experiments. *B*, uninduced or IFN- γ -treated (+ IFN γ) cells were detergent lysed, electrophoresed on 10% SDS-PAGE gels under reducing conditions, and transferred to nitrocellulose. Blots were stained for Ii with the mAb PIN1.1. Sweig and Jurkat cells are Ii⁺ and Ii⁻ cell lines, respectively. These data are from one of two to three independent experiments.

CD80 cells contain detectable Ii, although Ii expression is inducible in SUM159PT cells by a 48-h treatment with IFN- γ . Therefore, SUM159PT and Mel 202 tumor cells do not constitutively express Ii; therefore, Ii will not be present in the transduced vaccine cells to inhibit binding and presentation of endogenously synthesized peptides.

HLA-DRB0101 Transductants Stimulate HLA-DR Allogeneic PBMCs. Coculture of cells expressing functional HLA-DR molecules with allogeneic CD4⁺ T lymphocytes results in T-cell proliferation (27). Therefore, to determine whether the HLA-DRB0101 molecules expressed by the transduced tumor cell vaccines are functional, we cocultured the various transductants with allogeneic PBMCs. Responder non-HLA-DRB0101 PBMCs were mixed with various

numbers of irradiated transductants, and proliferation was assessed by measuring the SI at the end of 6 days of culture. Irradiated allogeneic PBMCs were used as a positive control. As shown in Fig. 5A, SUM/DR1/CD80 cells induce high SI, whereas SUM, SUM/CD80, or SUM/DR1 transductants produce only background levels. Therefore, the cell-based vaccines activate allogeneic PBMCs, provided they coexpress DRB0101 and CD80.

Transduced Tumor Cells Present an HLA-DR1-restricted TT Peptide. TT peptide p2 is an HLA-DR1-restricted epitope (22). If the HLA-DRB0101 molecules of the transductants are properly conformed and functional, when pulsed with the TT p2 peptide, the transductants should activate TT-specific HLA-DRB0101 lymphocytes. Because the TT-specific CD4⁺ T-cell precursor frequency in peripheral blood of the DRB0101 donor was low (data not shown), the HLA-DRB0101 PBMCs were boosted *in vitro* with TT to expand the number of TT-reactive T cells. TT-booster PBMCs were incubated at various ratios with tumor cell transductants pulsed with various quantities of TT p2 peptide to determine whether the transductants present this HLA-DR1-restricted epitope. T-cell activation was assessed by measuring IFN- γ release. As shown in Fig. 5B, SUM/DR1/CD80 tumor cells activate the TT-specific T cells as or more efficiently than EBV-transformed HLA-DR1 B cells (DR1-EBV B cells), whereas HLA-DR1-negative parental SUM cells do not activate. Therefore, SUM/DR1/CD80 tumor cells are effective APCs for an HLA-DR1-restricted epitope, further demonstrating that the transduced MHC class II molecules are functional.

HLA-DR1/CD80 Tumor Cell Transductants Present Endogenous TT and Activate TT-specific T Lymphocytes. We have generated the DR1/CD80 transductants to use as cancer vaccines to immunize patients and activate their T lymphocytes to tumor-encoded

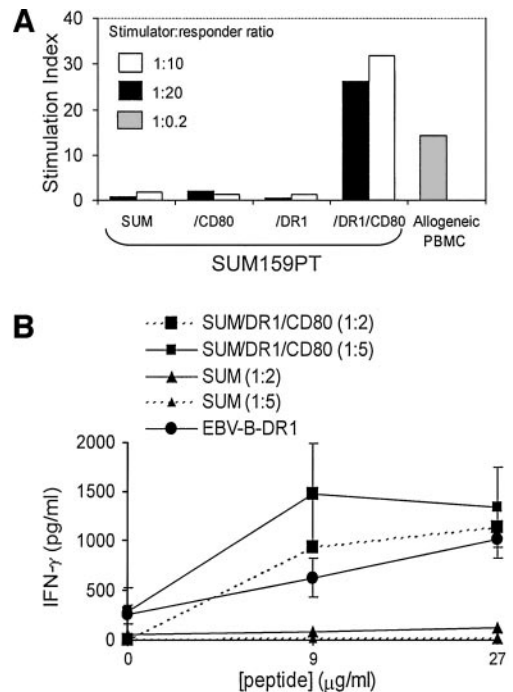


Fig. 5. SUM/DR1/CD80 cells induce proliferation of allogeneic T cells and present a DR1-restricted peptide to DRB0101 PBMC. *A*, irradiated SUM transductants expressing HLA-DRB0101 and/or CD80 or allogeneic PBMCs were cocultured with non-DRB0101 PBMCs at various ratios of APCs to responder lymphocytes. Proliferation was assessed by measuring the SI after 6 days of culture. These data are from one of three independent experiments. *B*, SUM, SUM/DR1/CD80, or DRB0101-expressing EBV B cells were pulsed with the DR1-restricted TT peptide, p2, and cocultured with TT-primed DRB0101 PBMCs at various ratios of APCs to PBMCs. Supernatants were harvested after 2 days of culture and tested by ELISA for IFN- γ . These data are from one of three independent experiments. *Bars*, SD.

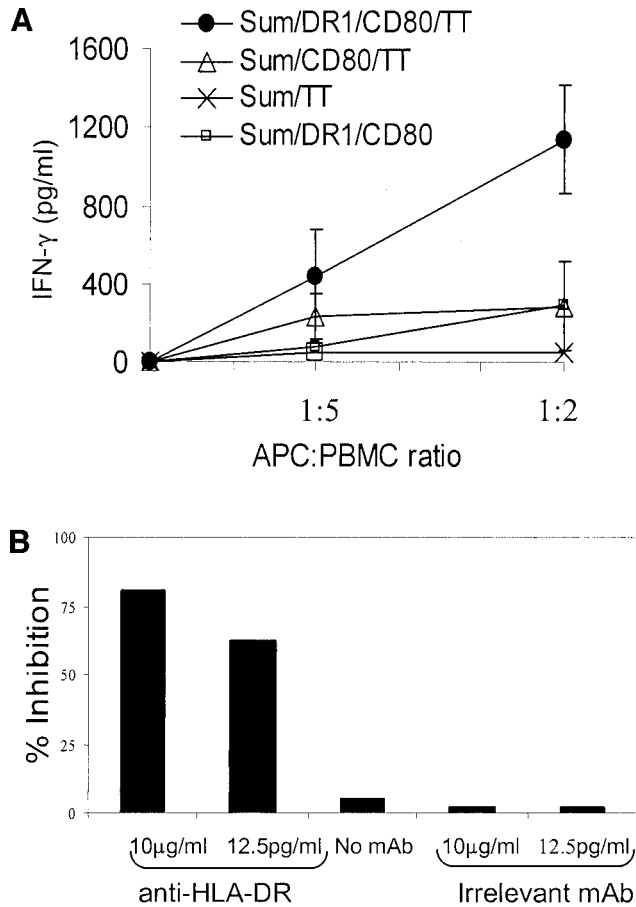


Fig. 6. SUM/DR1/CD80/TT cells activate HLA-DR-restricted DRB0101 PBMCs to tumor-encoded TT. *A*, irradiated SUM transductants were cocultured with TT-primed DRB0101 PBMCs at various ratios of APCs to responder PBMCs. Supernatants were harvested after 2 days of culture and tested by ELISA for IFN- γ . These data are from one of three independent experiments; bars, SD. *B*, HLA-DR-specific (L243) or irrelevant (28-14-8) mAb was added to culture wells containing irradiated SUM/DR1/CD80/TT cells before addition of TT-primed responder DRB0101 PBMCs at a ratio of 1:2 APCs to responder cells. Supernatants were harvested after 2 days of culture and tested by ELISA for IFN- γ . These data are from one of two independent experiments.

tumor peptides. To achieve this goal, the transductants must not only express functional HLA-DR molecules, but the DR molecules must also bind and present endogenously synthesized tumor peptides. To determine whether the transductants have this capability, we tested SUM/DR1/CD80/TT cells as APCs for endogenously encoded TT. Because the TT construct does not contain a signal sequence, TT protein will reside in the cytoplasm and serve as a “model” tumor antigen for a cytoplasmically localized tumor antigen.

Adherent cell-depleted HLA-DRB0101 PBMCs were boosted *in vitro* with TT as per the experiment of Fig. 5*B* and cocultured at various ratios with irradiated transduced SUM cells. Activation was assessed by measuring IFN- γ release. As shown in Fig. 6*A*, SUM/DR1/CD80/TT tumor cells activate a potent T-cell response, whereas SUM transductants without DRB0101 (SUM/CD80/TT), without TT (SUM/DR1/CD80), or without DRB0101 and CD80 (SUM/TT) do not activate. Because SUM/TT and SUM/CD80/TT cells do not activate, TT is not being released into the culture medium and being presented by other APCs in the PBMC population. Therefore, tumor cells transduced with *HLA-DRB0101*, *CD80*, and *TT* genes are effective APCs for endogenously encoded molecules.

To further analyze whether the presentation of endogenous TT is DR1 restricted, anti-HLA-DR mAb (L243) was added at various concentrations at the beginning of the assay. As shown in Fig. 6*B*, in

the presence of the highest dose of antibody, T-cell activation is inhibited >80%, whereas an irrelevant isotype-matched mouse H-2L^d-specific mAb does not inhibit.

DR1/CD80/TT Tumor Cells Activate CD4⁺ T Lymphocytes.

To identify the PBMCs that are specifically activated by the vaccine cells, adherent cell-depleted, TT-primed DRB0101 PBMCs were depleted for CD4⁺ or CD8⁺ T cells or for B cells and then used as responding cells in antigen presentation assays with SUM/DR1/CD80/TT transductants. T and B cells were depleted by magnetic bead separation. To ascertain the efficiency of the depletions, PBMCs before and after depletion were tested by flow cytometry for the percentage of CD4⁺, CD8⁺, and immunoglobulin⁺ (B) cells. As shown in Fig. 7*A*, antibody depletion eliminated 98–99% of the target lymphocytes. The relatively high percentage of CD4⁺ T cells and low percentage of CD8⁺ T cells in the undepleted, TT-boosted population probably reflects the preferential activation of CD4⁺ T cells during the *in vitro* boosting process.

After T- and B-cell depletion, the resulting PBMCs were cocultured with irradiated vaccine cells and endogenous TT presentation assessed by ELISA. As shown in Fig. 7*B*, CD4-depleted PBMCs stimulated with SUM159/DR1/CD80/TT vaccine cells are not activated, as measured by IFN- γ release. In contrast, CD8-depletion did not affect IFN- γ release. Likewise, depletion of CD19⁺ cells did not affect IFN- γ release, demonstrating that cross-priming by B cells is not occurring. Stimulation of undepleted PBMCs with SUM159/DR1/CD80 APCs also did not cause IFN- γ release, demonstrating that

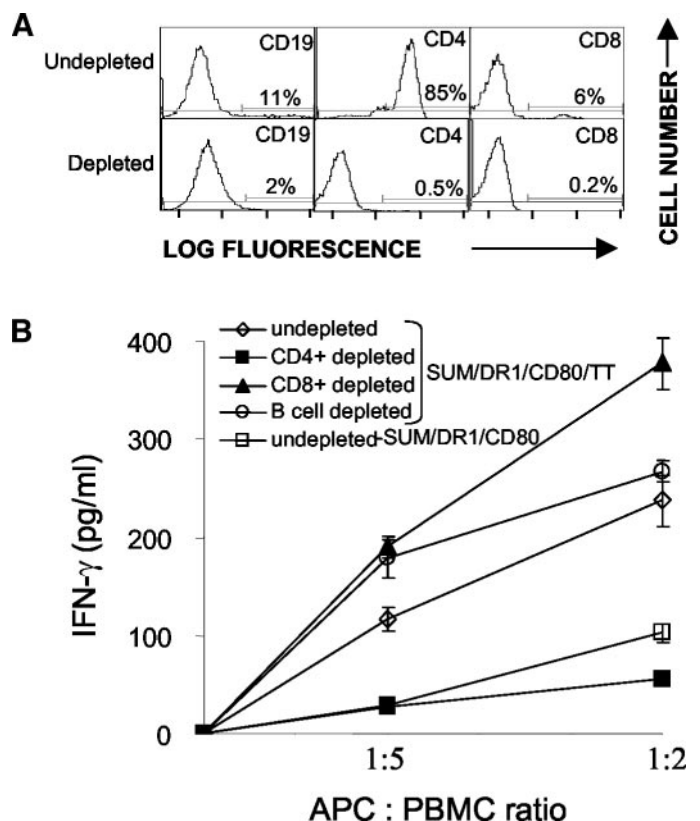


Fig. 7. SUM/DR1/CD80/TT cells activate CD4⁺ T lymphocytes to tumor-encoded antigen. *A*, DRB0101 PBMCs were primed *in vitro* with TT, and separate aliquots were depleted for CD4⁺, CD8⁺, or CD19⁺ cells. The resulting cells were stained by direct immunofluorescence for these populations. Values in the lower right-hand corners of each profile represent the percentage of the indicated cells. *B*, irradiated SUM/DR1/CD80/TT or SUM/DR1/CD80/transductants were cocultured with CD4, CD8, or CD19-depleted, or not depleted, TT-primed DRB0101 PBMCs at various ratios of APCs to responder PBMCs. Supernatants were harvested after 2 days of culture and tested by ELISA for IFN- γ . These data are from one of two to four independent experiments; bars, SD.

PBMC activation is TT specific. Therefore, CD4⁺ PBMCs are activated by the vaccine cells, and the activation is mediated by direct presentation of endogenously synthesized TT by the genetically modified tumor cell transductants.

DISCUSSION

Recent animal studies and some clinical trials have indicated that the use of genetically engineered tumor cells as vaccines may have therapeutic efficacy for the treatment of cancer (28–30). Parallel studies have recognized the critical role played by CD4⁺ T cells in orchestrating the host immune response against cancer and have developed methods to activate CD4⁺ T cells (2–5, 31–33). Because CD4⁺ T cells play a central role in enhancing antitumor immunity, our laboratory has focused on facilitating the activation of these cells. We have hypothesized that tumor cells that constitutively express MHC class I molecules do not contain Ii and are genetically modified to express syngeneic MHC class II molecules, and costimulatory molecules will function as APCs for endogenously synthesized MHC class I- and class II-restricted tumor antigen epitopes. If used as immunogens in tumor-bearing individuals, such cells will serve as “vaccines” to activate tumor-specific CD4⁺ and CD8⁺ T lymphocytes that will facilitate regression of wild-type tumor (2, 6). Because the efficacy of these vaccines against wild-type primary tumors and experimental and spontaneous metastatic disease has been demonstrated in multiple mouse models (8, 10, 11, 34, 35), the goal of this study was to translate this strategy for clinical use.

Activation of tumor-specific CD4⁺ T cells by the cell-based vaccines is based on the supposition that the MHC class II molecules of the vaccine cells bind peptides synthesized within the tumor cells and directly present these peptides to CD4⁺ T lymphocytes. This mode of presentation is different from that of professional APCs that typically bind peptides derived from endocytosed, exogenously synthesized antigens (36). This fundamental difference is attributable to the absence of the MHC class II-associated accessory molecule, Ii, in the vaccine cells. If APCs express Ii, Ii binds to newly synthesized MHC class II molecules, thereby preventing the binding of endogenously derived peptides and favoring the binding of exogenously synthesized peptides (37). However, in the absence of Ii, MHC class II molecules bind peptides derived from endogenously synthesized antigens (14). Because the MHC class II and Ii genes are coordinately regulated and coordinately induced by IFN- γ (38), professional APCs and tumor cells that constitutively express MHC class II genes and/or are induced by IFN- γ are unlikely to be APCs for endogenously synthesized tumor antigens. Studies with Ii⁺ and Ii⁻ MHC class II⁺ tumor cells support this concept and demonstrate that the most efficacious vaccines are MHC class II⁺Ii⁻ (14, 16, 17).

Early studies suggested that expression of MHC class II molecules without coexpression of Ii produces reduced levels of class II molecules that are improperly conformed and unable to function as antigen presentation elements (39–41). More recent studies have demonstrated that the Ii dependency of MHC class II molecules is allele specific (42, 43), and that many MHC class II alleles do not require Ii expression for stability or antigen presentation function (44). The studies reported here demonstrating efficient antigen presentation by MHC class II⁺Ii⁻ tumor cell vaccines add HLA-DR0101 to the list of MHC class II alleles whose expression and function are independent of Ii coexpression.

In addition to the absence of Ii for maximal vaccine efficacy, the studies reported here demonstrate that optimal vaccine activity requires coexpression of CD80 for delivery of a costimulatory signal. This observation agrees with extensive mouse and human studies showing the requirement for costimulation for optimal T-cell activa-

tion (reviewed in Ref. 26), as well as many studies that showed that CD80 expression facilitates tumor rejection (45–47).

Several lines of evidence support the hypothesis that the MHC class II tumor cell-based vaccines activate CD4⁺ T cells by direct antigen presentation of endogenously encoded tumor antigens, rather than by cross-priming or indirect presentation via host-derived APCs, as suggested by other investigators for other cell-based vaccines and/or tumor cells (48–50):

(a) If tumor-encoded antigens were presented by host-derived APCs such as B cells or other APCs in the PBMCs, then SUM/DR1/TT, SUM/TT, and SUM/CD80/TT cell lines should be just as effective APCs as are SUM/DR1/CD80/TT. However, only SUM/DR1/CD80/TT vaccine cells activate PBMCs.

(b) If professional APCs, rather than the tumor cell vaccines, are the relevant APCs, then removal of these professional APCs should eliminate T-cell activation. However, adherent cells (including dendritic cells and macrophages) are routinely removed from the PBMCs before their coculture with vaccine cells, and in some experiments, CD19⁺ B cells were also removed without affecting T-cell activation.

(c) Extensive *in vivo* studies using genetically marked vaccine cells conclusively demonstrated that the vaccine cells directly activate T lymphocytes (12–14). Therefore, it is unlikely that vaccine efficacy is attributable to leakage of tumor antigen, resulting in endocytosis by professional APCs for presentation by cross-priming.

The vaccines described here are based on the premise that tumor cells will be destroyed by CD8⁺ T cells with help from CD4⁺ T cells. Tumor-specific CD8⁺ T cells could be activated either by interacting with MHC class I/peptide complexes of the genetically modified vaccine cells or by cross-presentation of class I-restricted epitopes by professional APCs. In either case, the activated CD8⁺ T cells would be specific for MHC class I-restricted tumor peptides and for wild-type tumor cells. Although the vaccines described here are potent activators of CD4⁺ T cells, vaccine cell expression of a MHC class I allele shared with the patient's lymphocytes may facilitate an even stronger immune response by capitalizing on the close proximity of CD4⁺ and CD8⁺ T cells during their activation. A MHC class I allele could be expressed in the vaccines by retroviral transduction. Alternatively, for an allele such as HLA-A2, which is expressed by approximately 50% of the Caucasian population, an HLA-A2⁺ tumor cell line could be used as the “base” vaccine. Additional experiments assessing activation of CD8⁺ T cells by the vaccines generated in this study *versus* MHC class I-matched or -engineered vaccines will be necessary to address this issue.

A significant technical obstacle in generating the MHC class II cell-based vaccines has been to routinely achieve high level expression of the desired MHC class II alleles in human tumor cells. Because many human tumor cells and cell lines can be problematic to maintain in culture, standard transfection and electroporation techniques did not result in reproducible class II expression.⁵ In contrast, transduction using a bicistronic retrovirus encoding the DR α and DR β chain genes separated by an IRES routinely yielded high-level HLA-DR expression in a high proportion of transductants. The efficiency of the current retroviruses appears to be attributable to the placement of the DR α and DR β genes flanking the IRES, because a previous study using a retroviral construct encoding pig DQ α and DQ β genes run off of separate promoters and without an IRES produced only low-level, DQ-expressing cells (51). It is likely the IRES construct will be universally useful, because similar retroviruses encoding other HLA-DR alleles also reproducibly yield high-level MHC class II expression in additional human tumor lines.⁶

The potency of the MHC class II vaccines for activating CD4⁺ T cells

⁵ S. Dissanayake and J. Bosch, unpublished results.

⁶ J. Thompson and M. Pohl, unpublished results.

to tumor-encoded antigens suggests that these vaccines may have therapeutic efficacy for cancer patients. For example, the cell-based vaccines could be administered *in vivo* to patients with disseminated metastatic disease. Alternatively, they could be used *ex vivo* to activate patients' T cells for subsequent adoptive transfer. In either case, these vaccines provide a novel and potent approach for activating tumor-specific CD4⁺ T cells and merit further clinical development and testing.

ACKNOWLEDGMENTS

We thank D. Ilkovitch for making the pLXCX/CD80 virus and S. Sahni for preliminary work on the TT construct. We appreciate the following colleagues for providing various materials: Dr. E. Long (DR0101 plasmid), Dr. M. Tykocinski (CD80 plasmid), Drs. P. Fishmen and N. Fairweather (TT plasmid), Harvard Gene Therapy Institute (293T cells), Dr. D. Mann (PBMCs), Dr. M. Nishimura (EBV B cells), and Dr. K. McIntosh.

REFERENCES

- Ostrand-Rosenberg, S., Thakur, A., and Clements, V. Rejection of mouse sarcoma cells after transfection of MHC class II genes. *J. Immunol.*, *144*: 4068–4071, 1990.
- Ostrand-Rosenberg, S., Pulaski, B., Clements, V., Qi, L., Pipeling, M., and Hanyok, L. Cell-based vaccines for the stimulation of immunity to metastatic cancers. *Immunol. Rev.*, *170*: 101–114, 1999.
- Hung, K., Hayashi, R., Lafond-Walker, A., Lowenstein, C., Pardoll, D., and Levitsky, H. The central role of CD4⁺ T cells in the antitumor immune response. *J. Exp. Med.*, *188*: 2357–2368, 1998.
- Toes, R., Ossendorp, F., Offringa, R., and Melief, C. CD4 T cells and their role in antitumor immune responses. *J. Exp. Med.*, *189*: 753–756, 1999.
- Pardoll, D., and Topalian, S. The role of CD4⁺ T cell responses in anti-tumor immunity. *Curr. Opin. Immunol.*, *10*: 588–594, 1998.
- Ostrand-Rosenberg, S. Tumor immunotherapy: the tumor cell as an antigen-presenting cell. *Curr. Opin. Immunol.*, *6*: 722–727, 1994.
- Schoenberger, S. P., Toes, R. E., van der Voort, E. I., Offringa, R., and Melief, C. J. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature (Lond.)*, *393*: 480–483, 1998.
- Baskar, S., Glimcher, L., Nabavi, N., Jones, R. T., and Ostrand-Rosenberg, S. Major histocompatibility complex class II⁺B7-1⁺ tumor cells are potent vaccines for stimulating tumor rejection in tumor-bearing mice. *J. Exp. Med.*, *181*: 619–629, 1995.
- Ostrand-Rosenberg, S., Baskar, S., Patterson, N., and Clements, V. Expression of MHC class II and B7-1 and B7-2 costimulatory molecules accompanies tumor rejection and reduces the metastatic potential of tumor cells. *Tissue Antigens*, *47*: 414–421, 1996.
- Pulaski, B. A., Clements, V. K., Pipeling, M. R., and Ostrand-Rosenberg, S. Immunotherapy with vaccines combining MHC class II/CD80⁺ tumor cells with interleukin-12 reduces established metastatic disease and stimulates immune effectors and monokine induced by interferon γ . *Cancer Immunol. Immunother.*, *49*: 34–45, 2000.
- Pulaski, B. A., Terman, D. S., Khan, S., Muller, E., and Ostrand-Rosenberg, S. Cooperativity of *Staphylococcus aureus* enterotoxin B superantigen, major histocompatibility complex class II, and CD80 for immunotherapy of advanced spontaneous metastases in a clinically relevant postoperative mouse breast cancer model. *Cancer Res.*, *60*: 2710–2715, 2000.
- Armstrong, T., Clements, V., and Ostrand-Rosenberg, S. MHC class II-transfected tumor cells directly present antigen to tumor-specific CD4⁺ T lymphocytes. *J. Immunol.*, *160*: 661–666, 1998.
- Armstrong, T., Pulaski, B., and Ostrand-Rosenberg, S. Tumor antigen presentation: changing the rules. *Cancer Immunol. Immunother.*, *46*: 70–74, 1998.
- Qi, L., Rojas, J., and Ostrand-Rosenberg, S. Tumor cells present MHC class II-restricted nuclear and mitochondrial antigens and are the predominant antigen presenting cells *in vivo*. *J. Immunol.*, *165*: 5451–5461, 2000.
- Stumptner-Cuvelette, P., and Benaroch, P. Multiple roles of the invariant chain in MHC class II function. *Biochim. Biophys. Acta*, *1542*: 1–13, 2002.
- Clements, V. K., Baskar, S., Armstrong, T. D., and Ostrand-Rosenberg, S. Invariant chain alters the malignant phenotype of MHC class II⁺ tumor cells. *J. Immunol.*, *149*: 2391–2396, 1992.
- Armstrong, T., Clements, V., Martin, B., Ting, J. P.-Y., and Ostrand-Rosenberg, S. Major histocompatibility complex class II-transfected tumor cells present endogenous antigen and are potent inducers of tumor-specific immunity. *Proc. Natl. Acad. Sci. USA*, *120*: 123–128, 1997.
- Long, E. O., Rosen-Bronson, S., Karp, D. R., Malnati, M., Sekaly, R. P., and Jaraquemada, D. Efficient cDNA expression vectors for stable and transient expression of HLA-DR in transfected fibroblast and lymphoid cells. *Hum. Immunol.*, *31*: 229–235, 1991.
- Fairweather, N. F., Lyness, V. A., Pickard, D. J., Allen, G., and Thomson, R. O. Cloning, nucleotide sequencing, and expression of tetanus toxin fragment C in *Escherichia coli*. *J. Bacteriol.*, *165*: 21–27, 1986.
- Verbik, D. J., Murray, T. G., Tran, J. M., and Ksander, B. R. Melanomas that develop within the eye inhibit lymphocyte proliferation. *Int. J. Cancer*, *73*: 470–478, 1997.
- Kingston, R., Chen, C., and Okayama, H. Calcium phosphate transfection. In: R. Coico (ed.), *Current Protocols Immunology*, unit 10.13. New York: John Wiley & Sons, Inc., 2003.
- Panina-Bordignon, P., Tan, A., Termijtelen, A., Demotz, S., Corradin, G., and Lanzavecchia, A. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur. J. Immunol.*, *19*: 2237–2242, 1989.
- Brosterhus, H., Brings, S., Leyendeckers, H., Manz, R. A., Miltenyi, S., Radbruch, A., Assenmacher, M., and Schmitz, J. Enrichment and detection of live antigen-specific CD4⁺ and CD8⁺ T cells based on cytokine secretion. *Eur. J. Immunol.*, *29*: 4053–4059, 1999.
- Chicz, R. M., Urban, R. G., Gorga, J. C., Vignali, D. A., Lane, W. S., and Strominger, J. L. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *J. Exp. Med.*, *178*: 27–47, 1993.
- Demotz, S., Lanzavecchia, A., Eisel, U., Niemann, H., Widmann, C., and Corradin, G. Delineation of several DR-restricted tetanus toxin T cell epitopes. *J. Immunol.*, *142*: 394–402, 1989.
- Carreno, B. M., and Collins, M. The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. *Annu. Rev. Immunol.*, *20*: 29–53, 2002.
- Fabre, J. W. The allogeneic response and tumor immunity. *Nat. Med.*, *7*: 649–652, 2001.
- Whelan, M., Whelan, J., Russell, N., and Dalgleish, A. Cancer immunotherapy: an embarrassment of riches? *Drug Discov. Today*, *8*: 253–258, 2003.
- Mitchell, M. S. Cancer vaccines, a critical review. Part II. *Curr. Opin. Investig. Drugs*, *3*: 150–158, 2002.
- Pardoll, D. Cancer vaccines. *Nat. Med.*, *4*: 525–531, 1998.
- Kalams, S. A., and Walker, B. D. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J. Exp. Med.*, *188*: 2199–2204, 1998.
- Surman, D. R., Dudley, M. E., Overwijk, W. W., and Restifo, N. P. Cutting edge: CD4⁺ T cell control of CD8⁺ T cell reactivity to a model tumor antigen. *J. Immunol.*, *164*: 562–565, 2000.
- Cohen, P. A., Peng, L., Plautz, G. E., Kim, J. A., Weng, D. E., and Shu, S. CD4⁺ T cells in adoptive immunotherapy and the indirect mechanism of tumor rejection. *Crit. Rev. Immunol.*, *20*: 17–56, 2000.
- Pulaski, B., and Ostrand-Rosenberg, S. MHC class II and B7.1 immunotherapeutic cell-based vaccine reduces spontaneous mammary carcinoma metastases without affecting primary tumor growth. *Cancer Res.*, *58*: 1486–1493, 1998.
- Ostrand-Rosenberg, S., Pulaski, B., Armstrong, T., and Clements, V. Immunotherapy of established tumor with MHC class II and B7.1 cell-based tumor vaccines. *Adv. Exp. Med. Biol.*, *451*: 259–264, 1998.
- Pieters, J. MHC class II-restricted antigen processing and presentation. *Adv. Immunol.*, *75*: 159–200, 2000.
- Busch, R., Cloutier, I., Sekaly, R., and Hammerling, G. Invariant chain protects class II histocompatibility antigens from binding intact polypeptides in the endoplasmic reticulum. *EMBO J.*, *15*: 418–428, 1996.
- Mach, B., Steimle, V., Martinez-Soria, E., and Reith, W. Regulation of MHC class II genes: lessons from a disease. *Annu. Rev. Immunol.*, *14*: 301–331, 1996.
- Bikoff, E., Huang, L.-Y., Episkopou, V., Meerwijk, J., Germain, R., and Robertson, E. Defective major histocompatibility complex class II assembly, transport, peptide acquisition, and CD4⁺ T cell selection in mice lacking invariant chain. *J. Exp. Med.*, *177*: 1699–1712, 1993.
- Viville, S., Neeffjes, J., Lotteau, V., Dierich, A., Lemeur, M., Ploegh, H., Benoist, C., and Mathis, D. Mice lacking the MHC class II-associated invariant chain. *Cell*, *72*: 635–648, 1993.
- Elliott, E. A., Drake, J. R., Amigorena, S., Elsemore, J., Webster, P., Mellman, I., and Flavell, R. A. The invariant chain is required for intracellular transport and function of major histocompatibility complex class II molecules. *J. Exp. Med.*, *179*: 681–694, 1994.
- Bikoff, E. K., Germain, R. N., and Robertson, E. J. Allelic differences affecting invariant chain dependency of MHC class II subunit assembly. *Immunity*, *2*: 301–310, 1995.
- Kenty, G., and Bikoff, E. BALB/c invariant chain mutant mice display relatively efficient maturation of CD4⁺ T cells in the periphery and secondary proliferative responses elicited upon peptide challenge. *J. Immunol.*, *163*: 232–241, 1999.
- Rajagopalan, G., Smart, M., Krco, C., and David, C. Expression and function of transgenic HLA-DQ molecules and lymphocyte development in mice lacking invariant chain. *J. Immunol.*, *169*: 1774–1783, 2002.
- Baskar, S., Ostrand-Rosenberg, S., Nabavi, N., Nadler, L. M., Freeman, G. J., and Glimcher, L. H. Constitutive expression of B7 restores immunogenicity of tumor cells expressing truncated major histocompatibility complex class II molecules. *Proc. Natl. Acad. Sci. USA*, *90*: 5687–5690, 1993.
- Townsend, S. E., and Allison, J. P. Tumor rejection after direct costimulation of CD8⁺ T cells by B7-transfected melanoma cells. *Science (Wash. DC)*, *259*: 368–370, 1993.
- Chen, L., Linsley, P. S., and Hellstrom, K. E. Costimulation of T cells for tumor immunity. *Immunol. Today*, *14*: 483–486, 1993.
- Huang, A., Golumbek, P., Ahmadzadeh, M., Jaffee, E., Pardoll, D., and Levitsky, H. Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science (Wash. DC)*, *264*: 961–965, 1994.
- Robinson, B., Scott, B., Lake, R., Stumble, P., and Nelson, D. Lack of ignorance to tumor antigens: evaluation using nominal antigen transfection and T-cell receptor transgenic lymphocytes in Lyons-Parish analysis: implications for tumor tolerance. *Clin. Cancer Res.*, *7*: 2811–2817, 2001.
- Nguyen, L., Elford, A., Murakami, K., Garza, K., and Schoenberger, S. Tumor growth enhances cross-presentation leading to limited T cell activation without tolerance. *J. Exp. Med.*, *195*: 423–435, 2002.
- Shimada, H., Germana, S., Sonntag, K., Banerjee, P., Moore, D., Sachs, D., and Leguern, C. MHC class II α/β heterodimeric cell surface molecules expressed from a single proviral genome. *Hum. Gene Ther.*, *10*: 2397–2405, 1999.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Activation of Tumor-specific CD4⁺ T Lymphocytes by Major Histocompatibility Complex Class II Tumor Cell Vaccines: A Novel Cell-based Immunotherapy

Samudra K. Dissanayake, James A. Thompson, Jacobus J. Bosch, et al.

Cancer Res 2004;64:1867-1874.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/64/5/1867>

Cited articles This article cites 50 articles, 22 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/64/5/1867.full#ref-list-1>

Citing articles This article has been cited by 14 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/64/5/1867.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.