Tumor Cells Transduced with the MHC Class II Transactivator and CD80 Activate Tumor-Specific CD4+ T Cells Whether or Not They Are Silenced for Invariant Chain

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Abstract

The specificity and potency of the immune system make immunotherapy a potential strategy for the treatment of cancer. To exploit this potential, we have developed cell-based cancer vaccines consisting of tumor cells expressing syngeneic MHC class II and costimulatory molecules. The vaccines mediate tumor regression in mice and activate human CD4+ T cells in vitro. Previous vaccines were generated by transducing MHC II negative tumor cells with a single HLA-DR allele. Because expression of multiple MHC II alleles would facilitate presentation of a broader repertoire of tumor antigens, we have now transduced tumor cells with the MHC class II transactivator (CIITA), a regulatory gene that coordinately increases expression of all MHC II alleles. Previous studies in mice indicated that coexpression of the MHC II accessory molecule invariant chain (Ii) inhibited presentation of endogenously synthesized tumor antigens and reduced vaccine efficacy. To determine if Ii expression affects presentation of MHC class II–restricted endogenously synthesized tumor antigens, mice transduced with the CIITA, CD80 costimulatory molecule gene, and with or without small interfering RNAs (siRNA) specific for Ii. Ii expression is silenced >95% in CIITA/CD80/siRNA transductants; downregulation of Ii does not affect HLA-DR expression or stability; and Ii and Ii−transductants activate human CD4+ T cells to DRB1*0701-restricted HER-2/neu epitopes. Therefore, tumor cells transduced with the CIITA, CD80, and with or without Ii siRNA present endogenously synthesized tumor antigens and are potential vaccines for activating tumor-specific CD4+ T cells. (Cancer Res 2006; 66(2): 1147-54)

Introduction

Immunotherapy is a potential approach for the treatment and/or prevention of cancer because of its specificity, sensitivity, potency, and long-term memory. T lymphocytes, the cellular arm of the immune response, are particularly promising because they have the capability of localizing to tumor sites and directly killing tumor cells. Because of these characteristics, vaccines and/or immunotherapy may facilitate the destruction of existing disseminated metastatic tumor cells and protect individuals against the recurrence of primary tumors and/or the outgrowth of latent metastatic cells (1, 2).

T cells that are cytotoxic for tumor cells are typically CD8+ T lymphocytes, and optimal activation of these cells usually requires coactivation of CD4+ T helper lymphocytes (3, 4). CD4+ T lymphocytes are also required for generating CD8+ T memory cells (5–7). Because of these critical roles for CD4+ T cells, we are developing cancer vaccines that specifically target the activation of CD4+ T cells while concurrently activating cytotoxic CD8+ T lymphocytes.

CD4+ T lymphocytes are activated to peptide antigen that is presented by MHC class II molecules. Because MHC II molecule expression is usually limited to professional antigen-presenting cells (APC), immunity to most pathogens requires that professional APCs acquire antigen from exogenous sources. To facilitate the presentation of endocytosed antigen, professional APCs contain the MHC class II–associated accessory molecule, invariant chain (Ii). Ii hinders the presentation of endogenously synthesized peptides and favors the presentation of antigen acquired by endocytosis. It mediates this effect by binding to newly synthesized MHC class II molecules in the endoplasmic reticulum and preventing them from acquiring peptides of endogenously synthesized molecules. The Ii chain also contains trafficking signals, which guide newly synthesized MHC II molecules to the endocytic pathway where Ii protein is degraded and peptides derived from endocytosed proteins bind (reviewed by refs. 8, 9). In professional APCs, MHC class II and Ii molecules are coordinately regulated at the transcriptional level by the MHC class II transcriptional activator (CIITA), a master regulatory gene that controls expression of all MHC II alleles (10–13). This coordinate regulation ensures that professional APCs efficiently present antigenic peptides acquired from extracellular sources.

Ii has been considered essential for MHC II function. Its requirement is supported by the finding that Ii knockout mice have dysfunctional or very low levels of MHC class II molecules, and CD4+ T cell activation is minimal (14–18). In contrast, some Ii-negative nonprofessional APCs when transfected or transduced with MHC class II genes present antigen and activate CD4+ T cells (16, 19–22), suggesting that MHC class II molecules can be fully functional in the absence of Ii.

Based on the assumption that coexpression of Ii blocks endogenous antigen presentation, we have produced cancer vaccines by transducing MHC II tumor cells with syngeneic MHC II and costimulatory molecule genes. The vaccines mediate tumor regression in mice and activate tumor-specific CD4+ T cells (20, 23–26). Activated CD4+ T cells in both mouse and human systems are specific for antigens encoded by the vaccine cells. These vaccines have been produced by transducing MHC class II tumor cells with a single HLA-DR allele. Because expression of multiple
MHC II alleles may facilitate presentation of a broader repertoire of tumor antigens, we have now transduced tumor cells with the CIITA. To determine if li coexpression affects T-cell activation to endogenous antigen, we have introduced small interfering RNAs (siRNA) specific for li into human tumor cells transduced with the CIITA and CD80 costimulatory molecule genes. The transductants efficiently activate human CD4+ T cells to HER-2/neu tumor antigen epitopes, suggesting that this strategy may be useful for vaccine design. Properly confirmed and functional MHC II heterodimers are present in transductants with or without li siRNA, indicating that li is not essential for the HLA-DR function. Surprisingly, transductants with or without the li siRNA are equally efficient at activating CD4+ T cells, indicating that in this system, li does not impair endogenous antigen presentation.

Materials and Methods

Cells. SUM159PT, Jurkat, Sweg, 293T, and peripheral blood mononuclear cells (PBMC) were handled as described (20). The human breast cancer line MCF10CA1 (hereafter called MCF10) and its nonmalignant counterpart MCF10A were cultured in MCF10 medium (DMEM/Hams F12, 1:1; 5% heat-inactivated FCS; 0.029 mol/L Na bicarbonate; 10 mmol/L HEPES; ref. 27) or MCF10A medium supplemented with 10 μg/mL insulin, 0.5 μg/mL hydrocortisone, 100 μg/mL cholaer kinase (all from Sigma, St. Louis, MO), and 20 ng/mL epidermal growth factor (Invitrogen, Carlsbad, CA). MCF10 transductants were supplemented with puromycin (0.3 μg/mL; Clontech, Palo Alto, CA) or hygromycin (150 μg/mL; Calbiochem, San Diego, CA). The OMM2.3 human ocular melanoma line (28) was grown in RPMI with 10% heat-inactivated FCS and 5 × 10^{-5} mol/L β-mercaptoethanol. All cell lines and procedures were approved by the institutional review boards of the participating institutions.

siRNA. Complementary sequences in the coding region of the human li gene (Genbank accession no. NID NM_004355) were identified using the Ambion siRNA target finder search engine (Ambion, Inc., Austin, TX). Sequences with no homology to other known human mRNAs were chosen at random from the 3’ to 5’ end of the li mRNA. siRNAs were produced by in vitro transcription with T7 RNA polymerase (29) using the Ambion Silencer siRNA Construction kit and were made homologous to sequences 4, 8, 16, 24, and 50 (double adenine regions found by the Ambion siRNA’s “analyzing” program). They were inserted into the pSIREN Retro-Q vector (Clontech) according to the manufacturer’s directions. The forward and reverse primers were annealed and ligated to the linearized pSIREN Retro-Q vector with BamHI and EcoRI “sticky ends.”

Retroviral constructs, transductions, and drug selection. The human CIITA gene was cloned from cdNA1-amp tag/CIITA (30) into Litmus28 (New England Biolabs, Beverly, MA) using XbaI and EcoRI and then cloned into a modified pLNCX retroviral vector, pLNCX2(AvrII) (neo resistance; ref. 20) using RglII and AvrII.

The HLA-DRB1*0701 cDNA (in RSV5 vector; ref. 31) contained two point mutations: a guanine instead of an adenine at base 191. These errors were corrected using Splicing by Overlapping Extensions (SOEing; ref. 32) using the following four primers of a thymine at base 191. These errors were corrected using Splicing by Overlapping Extensions (SOEing; ref. 32) using the following four primers.

Overlapping Extensions (SOEing; ref. 32) using the following four primers:

- primer 1, AGTACCCGGGATGGTGTGCTTGAGACCTCCCTG; primer 2, AGGCCGAGAATCTCGCTCTGTTATAGAA; primer 3, TCTTATATACAGCGAGGATTGCTGCTGCT; primer 4, TAGTGCGTGCTGCTAGGCTGACTGGT.
- Reaction 1: 10 pmol/L RSV5/DRB1*0701 template and 0.5 μmol/L of primers 1 and 2; cycle at 95°C for 2 minutes, then 30 cycles of 95°C for 30 seconds, 60.2°C for 30 seconds, 72°C for 1 minute, then 72°C for 10 minutes. Reaction 2: 10 pmol/L RSV5/DRB1*0701 template and 0.5 μmol/L primers 1 and 2; same as PCR1, but annealing temperature was 62.3°C. Reaction 3: 10 pmol/L of product from reactions 1 and 2 were mixed with primers 1 and 4 and incubated at 95°C for 2 minutes followed by five rounds of 95°C for 30 seconds followed by 60.2°C for 30 seconds followed by 72°C for 1 minute. Then five rounds more of the same reaction with annealing temperature at 62.3°C followed by 23 rounds of the same reaction at 64.8°C followed by 72°C for 10 minutes. All reactions used 2 units of PFU turbo polymerase (Invitrogen) according to the manufacturer’s specifications. These primers added the Xhol and NotI restriction sites on the 5’ and 3’ ends of the cDNA, respectively. The corrected sequence was confirmed by sequencing of both strands.

Using the same restriction sites as for cloning of pLNCX2/HLA-DRB1, the HLA-DRB1*0701 or HLA-DRB1*0401 genes were cloned into the downstream site of the pRES/DRSA1001 vector containing the HLA-DRB1*0101 gene in the upstream site. The DRA0101-IRES-DRB1 section was excised from the resulting vector and cloned into the retroviral vector pLNCX2(AvrII). The pLNCX/CD80 retroviral construct, retrovirus production, and transductions were previously described (20).

Transduced cells were selected as follows: CD80 transductants (150 μg/mL hygromycin); DR4, DR7, and CIITA transductants (300 μg/mL G418); siRNA transductants (0.3 μg/mL puromycin). If 2 to 3 weeks of drug selection did not yield homogeneous populations of transgene-expressing cells, the transductants were sorted by magnetic bead selection (Miltenyi, Auburn, CA) according to the manufacturer’s directions.

Peptides, antibodies, reagents, and immunofluorescence. HER-2/neu peptide 98-114 (RLIRIVRGTQFLEDNYAL) and peptide 776-790 (GVGPSYVSRLLIGC; refs. 33, 34) were synthesized at the University of Maryland Biopolymer Laboratory. Monoclonal antibodies (mAb: HLA-DR-FITC and CD80-PE), streptavidin-PE, FITC-isotype, and PE-isotype controls were from BD Pharmingen (San Diego, CA). Biotinylated HLA-DR mAb (BIH0126) was from One Lambda, Inc. (Canoga Park, CA); HLA-DQ-PE and HLA-DP-FITC were from Chemicon (Temecula, CA); rat anti-mouse IgG-FITC was from ICN (Costa Mesa, CA); c-neu (Ab-2) was from Oncogene (Cambridge MA); CD4-FITC, CD8-FITC, and anti-human IgG-FITC were from Miltenyi Biotech; and human IgG-FITC was from Cappel (West Chester, PA). Culture supernatants of hybridomas W6/32 (pan HLA-A,B,C), L243 (pan anti-HLA-DR), PIN1.I (anti-Ii), and 28.14.8 (anti-H-2D^d) were prepared, and tumor cells and PBMCs were stained for cell surface markers (MHC class I, class II, CD80, CD8, and immunoglobulin) or fixed and stained for li as described (20).

Western blots. Western blots were done as described (20). Blots for MHC II were done as for li with the following modifications: cell lysates were loaded onto SDS-PAGE gels using nonreducing loading dye [0.2% SDS, 20% glycerol, 1.25 mol/L Tris (pH 6.8), and 0.4 mg/mL bromophenol blue]. Half of each sample was boiled for 5 minutes immediately before loading. Blocking buffer was 2% bovine serum in TBST. Membranes were incubated with undiluted supernantant from hybridoma L243, and the last wash was done for 1 hour.

T-cell priming. PBMCs from healthy donors (2 × 10^7/4 mL/well) were cultured in PBMC medium [Isco’s modified Dulbecco’s medium, 10% FCS, 1% penicillin, 1% streptomycin (BioSource, Rockville, MD), 2 mmol/L Glutamax (Bethesda Research Laboratories/Life Sciences, Grand Island, NY)] with 2 μg/mL of HER2 p98 or p776 in six-well tissue culture plates at 37°C and 5% CO2 for 5 days. Nonadherent cells were harvested, washed twice with PBMC medium, and replated in 24-well plates with 20 units/mL of recombinant human interleukin 2 (IL-2; R&D Systems, Minneapolis, MN) at 1 × 10^5/2 mL/well. HER2-activated nonadherent cells were harvested 7 days later; live cells were isolated using Histopaque-1077, cultured 1 to 5 days without exogenous IL-2, and used the following day. For some experiments, after incubation with IL-2, nonadherent PBMCs were cultured at 1 × 10^5/4 mL/well with 8 × 10^5, 50 Gy-irradiated SUM/D8/CD80 cells for 5 days, washed and cultured as above with IL-2 for 7 days, and washed and rested for 1 day before use.

Alternatively, PBMCs were obtained from HER-2/neu-immunized patients with stage III or IV breast, ovarian, or non–small cell lung cancer participating in a University of Washington Food and Drug Administration–approved phase I trial (35). Patients were immunized intradermally once a month for 6 months to the same regional draining lymph node site with three different peptides derived from HER-2/neu and adixed with 100 μg granulocyte macrophage colony-stimulating factor. PBMCs were
collected 1 month after the last immunization and cryopreserved. For ex vivo boost, PBMCs were thawed at 37 °C, washed twice, and resuspended at 3 x 10^6/mL in X-VIVO media [10% human AB serum, 2 mmol/L l-glutamine, 20 mmol/L HEPES buffer, and 10 mmol/L acetylcysteine solution (USP)]. The cells were stimulated with 10 mg/mL of HER-2/new peptides (p98, p776, or p98+p776) and incubated at 37 °C in 5% CO2 for 12 days. On days 4/5 and 8, 10 units/mL of recombinant human IL-2 (Chiron Corp., Emeryville, CA) and 10 ng/mL of recombinant human IL-12 (R&D System) were added. On day 12, the cells were harvested, washed, counted, tested by flow cytometry and enzyme-linked immunospot (ELISPOT), and resuspended at 1 x 10^6/mL in fresh media containing 1 x 10^6/mL of anti-CD3/CD28-coated beads to a final concentration of 10 or 100 bead(s) per T cell. Between days 14 and 23, the cell concentration was evaluated every 2 to 3 days, and the cells were diluted to 0.5 to 1 x 10^6/mL with fresh media as needed. On days 15, 18, 20, and 22, IL-2 was added to a final concentration of 30 units/mL, and on day 25, the expanded cells were harvested, washed, counted, and evaluated by flow cytometry and ELISPOT (36).

Antigen presentation assays. Antigen presentation assays and T-cell depletions were done as described (20) using Miltenyi human CD4 and CD8 beads with the following modifications: stimulator cells were used at 2.5 x 10^6 per well. MCF10-derived and MCF10A stimulator cells were not irradiated; all other stimulators were 50 Gy irradiated. Antibody blocking experiments included 20 µg/mL L243 (anti-HLA-DR), W6/32 (anti-class I MHC), or 28.14.8. For exogenous HER2 peptide presentation, assays were as for endogenous antigen presentation, except soluble HER2 peptide p98 or p776 was included at 2 µg/mL.

HLA-DR nomenclature and genotypes. Normal donor PBMCs are A24, A29, B44, B33, DR7, DR11, and DRB3.4. SUM159PT cells are A2, A24, B5, B15, DR4, and DR13. OMM2.3 cells are A11, A29, B7, and B52. MCF10 and MCF10A cells are A33, B55, B22, DR7, and DR4. HLA genotypes were determined by PCR typing and are referred to by their short-hand form (e.g., HLA-DR7 is DRB1*0701).

Statistical analyses. Means, SDs, and statistical significance as measured by Student’s t test were calculated using Excel v2002.

Results

siRNA down-regulates Ii. The human breast cancer epithelial cell line MCF10 (27), which does not constitutively express MHC class II or Ii molecules, was transduced with a retrovirus encoding the human CIITA gene (Fig. 1) and selected by magnetic bead sorting for MHC class II expression. CIITA-transduced MCF10 (MCF10/CIITA) cells are MHC class II (HLA-DR, DP, and DQ) and Ii positive as shown by immunofluorescence (Fig. 1B). Retroviruses expressing siRNAs for Ii were constructed to down-regulate Ii in MCF10/CIITA cells. Four sequences in the human Ii gene, starting with AA and having low GC content, were selected (sequences 4, 38, and 50). Double-stranded RNA molecules of these sequences were prepared and were transiently transfected into 293T cells that had previously been transduced with the CIITA retrovirus. Sequences 4 and 50 but not 38 or 16 down-regulated Ii by 4- to 50-fold (data not shown). To obtain stable transductants, sequence 4 was cloned into the pSIREN vector with two different termination signals giving vectors 4.1 and 4.2, with 4.1 containing additional sequence following the six thymines. Sequence 50 was not used because it contained a four-thymidine repeat that is a stop of transcription for the U6 polymerase III promoter. Additional sequences starting with AA and having low GC content were selected adjacent to sequence 50 and were inserted into pSIREN (sequences 48, 53, and 54). An additional sequence 32 was also randomly selected and inserted into pSIREN. Sequences were inserted using the forward and reverse primers shown in Fig. 1C. Retroviruses containing these six siRNAs were prepared and used to transduce MCF10, MCF10/CIITA, and CD80-expressing cells (MCF10/siRNA, MCF10/CIITA/siRNA, MCF10/CIITA/CD80/siRNA). Transduced cells were analyzed by flow cytometry 3 days after transduction. Lines containing siRNAs 32, 53, and 48 showed a marked down-regulation of Ii but not complete loss, whereas cells containing siRNAs 4.1, 4.2, and 54 had minimal down-regulation of Ii (data not shown).

Western analyses for Ii (mAb PIN1.1) were done 3 days after transduction to confirm that Ii expression was down-regulated. The predominant form of Ii in MCF10/CIITA cells is p35, with a smaller amount of p33 (Fig. 2A). The p35 isoform, which is translated via an alternative translation initiation site, is normally the less abundant isoform of Ii (37). Others have noted an increase in p35 in tumors (38). The p35 and p33 isoforms are both down-regulated >95% in the siRNAs 53, 48, or 32 transductants, and there is a slight down-regulation in siRNA 4.1. siRNAs 4.2 and 54 do not affect Ii expression. Interestingly, at 3 days after transduction, all of the down-regulated cell lines contain a 23-kDa band that corresponds to an Ii degradation product. To ascertain if p23

![Image](image-url)
CD80/CIITA/siRNA 53 and reduced >95% in lines 32 and 48 and non-siRNA transductants (Fig. 2). siRNA transduction. HLA-DR expression is the same in siRNA transductants were analyzed by immunofluorescence 3 weeks after cell surface expression of MHC class II molecules, the trans-

- 48 kDa that correspond to stable MHC class II heterodimers. Nonboiled samples contain bands migrating at 55 kDa that are not visible in the boiled samples. 3. Western blots of parental and transduced MCF10 cells probed for Ii with the PIN1.1 mAb. 

**Figure 2.** Ii siRNAs silence Ii expression in CIITA-transduced cells without altering HLA-DR expression. MCF10 cells were transduced with retroviruses containing the CIITA and siRNAs for Ii. A and B, Western blots of MCF10, transduced MCF10, and control Sweig and Jurkat cells probed for Ii with the PIN1.1 mAb. C and D, parental and transduced MCF10 cells stained for HLA-DR (mAb L243) or Ii (mAb PIN1.1). D, Western blots of parental and transduced MCF10 cells probed for HLA-DR (mAb L243) after 33 kDa that is subsequently endocytosed, PBMCs were mixed with supernatants from MCF10/CIITA/siRNA 32 cells. PBMCs were pulsed with p98 as a positive control. Peptide-pulsed PBMCs were cocultured with transduced tumor cells and IFN-γ production was measured. Soluble peptide p98 or p776 was added to some wells to determine if the trans-

**To determine if the CIITA/siRNA cells present endogenously synthesized tumor peptides, HER-2/neu peptide p98 and/or p776-activated T cells were cocultured with transduced tumor cells, and IFN-γ production was measured. Soluble peptide p98 or p776 was added to some wells to determine if the trans-

**To rule out that PBMCs were responding to secreted HER-2/neu that is subsequently endocytosed, PBMCs were mixed with supernatants from MCF10/CIITA/siRNA 32 cells. PBMCs were pulsed with p98 as a positive control. Peptide-pulsed PBMCs were cocultured with transduced tumor cells, and IFN-γ production was measured.
produced IFN-γ; however, supernatant-pulsed cells did not (data not shown). Therefore, MCF10/CIITA/CD80/siRNA 32 cells are presenting endogenously synthesized molecules.

Peptides p98 and p776 also activate CD8+ T cells, suggesting that they may contain nested MHC class I epitopes (33, 34). The HER-2/neu-activated T cells share DR7 with the transduced MCF10 cells, and DR7 and A24 with SUM159PT/DR7/CD80 cells. No MHC class I alleles are common between p98- and p776-activated PBMCs and transduced MCF10 cells. Because peptides p98 and p776 are presented by both DR4 and DR7, there is the potential for the activation of both CD4+ and CD8+ T cells by SUM159PT/DR7/CD80 but not by MCF10/CIITA/CD80/siRNA 32 cells. To identify which T cells are activated, PBMCs were primed with p98 and p776 and subsequently incubated with vaccine cells in the presence of antibodies to MHC class I and/or MHC class II. Antibodies to MHC class II block antigen presentation by MCF10/CIITA/CD80/siRNA 32 and MCF/CIITA/CD80, whereas antibodies to both MHC I and II block antigen presentation by SUM159/DR7/CD80 cells (Fig. 4C). To confirm the activation of CD4+ and CD8+ T cells, PBMCs were primed with p776 and depleted for CD4+ or CD8+ T cells, before activation by MCF10 or SUM159PT transductants. Depletion of CD4+ T cells completely eliminates T-cell activation by both MCF10/CIITA/CD80/32 and SUM/DR7/CD80 cells. Depletion of CD8+ T cells significantly reduces T-cell activation by SUM159PT transductants and has a smaller effect on MCF10/CIITA/CD80/32-induced T-cell activation (Fig. 4D and E). This latter effect is probably nonspecific because MCF10/CIITA/CD80/32 cells do not share MHC class I alleles with PBMCs. Similar results were obtained with p98-primed PBMCs (data not shown).

MCF10/CIITA/32 cells were also included in this experiment (Fig. 4D) to determine if coexpression of CD80 enhances boosting of HER-2/neu-specific CD4+ T cells. In agreement with earlier findings (20), CD80-expressing transductants are better stimulators. Therefore, the transductants activate both CD4+ and CD8+ T cells if they share common alleles with the responding PBMCs, and coexpression of CD80 enhances activation.

Nonmalignant cells do not activate T cells. A potential problem with cell-based vaccines is that they will activate T cells against nonmalignant cells due to cross-reactivity with normal self-antigens. To determine if the MHC II vaccines induce reactivity against nonmalignant cells, PBMCs were activated with HER-2/neu p776 peptide and tested on MCF10 cells and their nonmalignant counterpart, MCF10A cells. As measured by flow cytometry, MCF10A cells express HER-2/neu, although at slightly lower levels than MCF10 cells (Fig. 5A compare with Fig. 3A). Unlike MCF10 cells, MCF10A cells do not express MHC II molecules; however, they are inducible for MHC II if incubated for 48 hours with 1,000 units/mL of rIFNγ. As seen in Fig. 5B, neither untreated nor IFNγ-treated MCF10A cells activate T cells. Therefore, tumor-specific T cells are activated by MHC II+CD80+ tumor cell–based vaccines and not by nonmalignant cells of the same tissue origin.

Discussion

A goal of tumor immunotherapy is to activate T lymphocytes to tumor-encoded antigens. Although some tumor peptides have been identified, many are unknown, and it is unclear how diverse an immune response is needed to eradicate tumor cells in vivo.

![Figure 3.](image-url) MCF10, OMM2.3, and SUM159PT cells overexpress HER-2/neu and transduced HLA genes and do not express Ii. A, parental and transduced MCF10 and control NIH3T3 cells labeled with antibodies to HER-2/neu or CD80 and analyzed by flow cytometry. B, HLA-DR4 and HLA-DR7 retroviral constructs. C, parental and transduced OMM2.3 and SUM159 cells stained with antibodies to HLA-DR (mAb L243), Ii (mAb PIN1.1), HER-2/neu, CD80, or HLA-A,B,C and analyzed by flow cytometry. Untilled peaks, specific antibody-stained cells; filled peaks, isotype control stained cells.
Our vaccine design was based on the hypothesis that coexpression of Ii would inhibit the presentation of endogenously synthesized tumor peptides, a hypothesis supported by our own earlier work and extensive work of others in nontumor systems (8, 9). Recent mass spectroscopy studies (42) provide direct biochemical support for this hypothesis and also provide an explanation for the lack of inhibition of Ii for HER-2/neu peptides p98 and p776. These investigators showed that the MHC II molecules of MHC II−Ii− cells contain peptides presented by MHC II−Ii+ cells plus additional novel peptides, which are not presented by MHC II−Ii+ cells (42). Because p98 and p776 were originally identified as epitopes presented by MHC II−Ii+ professional APCs (33, 34), they are most likely in the category of epitopes that are presented by both Ii+ and Ii− APCs. However, the findings of ref. (42) make it likely that the MHC II−Ii+ transductants also present novel tumor antigen epitopes that are not presented by professional APCs. In this fashion, Ii RNAi vaccines may activate a more diverse repertoire of tumor-specific CD4+ T cells than professional APCs and may activate T cells that have not previously been tolerized by the tumor.

Clinical studies also support an inhibitory role for Ii. Chamuleau et al. have shown that acute myelogenous leukemia (AML) patients in complete remission whose HLA-DR+ myeloid leukemic blasts have low levels of the MHC class II–associated Ii peptide (CLIP), a degradation product of Ii, have a significantly better clinical prognosis than patients whose blasts are DR+CLIP+ (43). Similar to AML blasts of progressor patients, DM-deficient mice also have DR+CLIP+ APCs, which are inefficient presenters of endogenously synthesized molecules (44). Preferential expression of the Ii p35 isoform is also associated with increased malignancy in chronic lymphocytic leukemia, and this effect has been attributed to reduced presentation of endogenously synthesized...
tumor antigens (38). Expression of CLIP is also associated with polarization towards a type 2 CD4 (Th2) response (45, 46), which may favor tumor progression (47).

Although early studies suggested that li expression was essential for MHC class II function (14, 15, 17), there are now many reports showing that MHC II alleles are properly conformed and functional in the absence of li (19, 20, 25, 48, 49). The present report extends this conclusion and shows that peptide affinity for MHC II is not affected by li, because peptide binding to surface MHC II molecules is similar for li and li cells. Therefore, although li may be required during development for expression of some mouse MHC II alleles, most MHC II alleles are stable and functional in the absence of li.

We envision that the vaccine strategy described here will be used to generate MHC II allele–specific vaccines from established cell lines. We propose a “cocktail” approach in which a patient will be treated with a mixture of multiple cell lines expressing MHC class I and II molecules matched to their genotype. Following HLA typing, a patient’s “semicustomized cocktail” would be prepared from stocks of frozen transduced cells. This approach depends on the existence of shared tumor antigens and eliminates the need for autologous tumor cells, making it feasible to treat most patients. Although retroviruses could be used to induce MHC II and CD80 molecules, alternative techniques that are less controversial would be preferable.

This vaccine strategy has the potential to activate T cells to self-antigens that are also expressed on nonmalignant cells. Autoimmunity has not been observed in the three mouse tumor systems studied in vivo, and the absence of reactivity with the nonmalignant breast line MCF10A suggests that autoimmunity against normal cells will also not be a problem in patients. In addition, a DR4/DR7 patient with advanced metastatic ovarian melanoma has been treated with irradiated OMM2.3/CD80/DR4 and OMM2.3/CD80/DR7 vaccines, and no autoimmune or other complications were noted.

Vaccines consisting of tumor cells transduced with the CIITA and li siRNAs have several potential advantages over previous vaccines in which MHC II nonexpressing tumor cells were transduced with individual MHC II alleles. Vaccines made by transducing single HLA-DR alleles are limited to presenting tumor antigen epitopes restricted by the transduced allele(s). In contrast, CIITA-transduced vaccine cells express multiple HLA-DR alleles, as well as DP and DQ alleles, and hence have the potential to present a much broader repertoire of tumor antigen epitopes. The CIITA/siRNA vaccines also differ from the previous MHC II vaccines in that expression of the CIITA up-regulates accessory molecules, such as HLA-DM. Although our previous studies have not shown that HLA-DM expression facilitates vaccine efficacy (50), studies by others have shown that HLA-DM expression stabilizes MHC II in the absence of li, aids MHC II traffic, and helps edit the MHC II peptide repertoire (42).

The CIITA/li siRNA strategy also expands the choice of tumor cells which could be used to generate vaccines to include tumors that constitutively express MHC II or are inducible for MHC II by treatment with IFNγ. Tumor cells that constitutively coexpress MHC II and costimulatory molecules, such as some leukemias (43), are particularly attractive targets for li siRNA therapy because creating a vaccine would only require down-regulating li via RNA interference (RNAi). This approach is supported by studies in which li was down-regulated in MHC II–positive, li-positive mouse tumor cells by antisense RNA (51–55). Mice immunized with the li antisense down-regulated tumor cells were protected against later challenge with wild-type tumor. Because siRNA is more effective in down-regulating li than antisense RNA, li siRNA vaccines may have more therapeutic efficacy than li antisense vaccines. Therefore, tumor cells expressing the CIITA and costimulatory molecules may be useful reagents, and concomitant down-regulation of li via RNAi may further improve vaccine efficacy and protect and/or treat tumor recurrence and/or metastatic disease.

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Tumor Cells Transduced with the MHC Class II Transactivator and CD80 Activate Tumor-Specific CD4+ T Cells Whether or Not They Are Silenced for Invariant Chain

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