Turning tumor cells in situ into T-helper cell-stimulating, MHC class II tumor epitope-presenters: immuno-curing and immuno-consolidation

Gilda G. Hillman, Nikoletta L. Kallinteris, Xueqing Lu, Yu Wang, Jennifer L. Wright, Yu Li, Shuzhen Wu, Jeffrey D. Forman, Joseph V. Gulfo, Robert E. Humphreys, Minzhen Xu,*

Department of Radiation Oncology, Barbara Ann Karmanos Cancer Institute at Wayne State University School of Medicine, 4100 John R., Detroit, MI 48201, USA

Antigen Express Inc., 100 Barber Avenue, Worcester, MA 01606-2478, USA

Summary

Immunological control or cure of tumors depends on initiating a robust T helper cell response to MHC class II epitopes of tumor-associated antigens. T helper cells regulate the potency of cytotoxic T lymphocyte and antibody responses. We have developed a novel approach to stimulate T helper cells by converting tumor cells into MHC class II molecule-positive, antigen-presenting cells. Furthermore, using antisense methods, we suppress expression of the Ii protein, that normally blocks the antigenic peptide binding site of MHC class II molecules during synthesis in the endoplasmic reticulum. In such gene-engineered tumor cells, the MHC class II molecules pick up antigenic peptides, which have been transported into the endoplasmic reticulum for binding to MHC class I molecules. All nucleated cells create such “surveys of self” to detect viral or malignant transformation. Our method extends that survey of self to MHC class II endogenous tumor-associated antigens. Simultaneous presentation of tumor antigens by both MHC class I and II generates a robust and long-lasting antitumor immune response. Injecting murine tumors with genes, which induce MHC class II molecules and suppress Ii protein, cures a significant number of animals with renal and prostate tumors. We have developed analogous human gene vectors that are suitable for most patients and cancers, because they are monomorphic and active in all HLA-DR alleles. We review our findings, and analyze remaining issues for preclinical study and the design of clinical trials.

© 2003 Elsevier Ltd. All rights reserved.

KEYWORDS

Immunotherapy; Cancer vaccine; MHC class II; T helper cells

Introduction

By inducing major histocompatibility complex (MHC) class II molecules on tumor cells in situ and suppressing the immunoregulatory Ii invariant
chain protein, we have developed a potent tumor cell autovaccine therapy for many tumors.\textsuperscript{1–4} This method uses simple gene-regulating reagents potentially usable in all patients, regardless of histotype.

Most tumors are MHC class II molecule-negative and cannot directly stimulate CD4\(^+\) T helper cells, which otherwise would up-regulate cytotoxic T lymphocyte (CTL) and antibody responses against the tumor. We induce MHC class II molecules in the tumor cells by transfecting genes for MHC class II transactivator (CIITA) or interferon-gamma (IFN-\(\gamma\)). However, we must then suppress the co-induced immunoregulatory protein Ii by antisense methods. The normal function of the Ii protein is to block the antigenic peptide binding site of MHC class II molecules at synthesis in the endoplasmic reticulum (ER), until the proteolytic release of Ii occurs in a post-Golgi antigenic peptide charging compartment. By suppressing the Ii protein in the ER, nascent MHC class II molecules bind peptides transported there for binding to MHC class I molecules. In such cells, tumor peptides, which have been processed and transported into the ER, are bound to and presented by both "unblocked" MHC class II molecules and MHC class I molecules (Fig. 1).

Induction of such MHC class II-positive, Ii-suppressed tumor cells leads to presentation of a large repertoire of T helper cell-recognized epitopes, with no need to identify each patient’s MHC class II histotypes. In mice, this therapy induces both T helper and CTL responses, curing established tumors. This autovaccine therapy protects against subsequent challenge with the same tumor but not another unrelated syngeneic tumor. Our genetically controlled immunotherapy needs to transform only a fraction of the cells in a treated nodule, in order to establish a potent, systemic immune response capable of eradicating non-transduced tumor cells.

Intratumoral gene transfections, with adenovirus vectors or DNA plasmid vectors delivered in liposomes, work well in murine models of renal cell and prostate adenocarcinomas. Human reagents are being developed for clinical trials. This immunotherapy is augmented with low levels of IL-2, IFN-\(\gamma\) cytokine genes and radiation. We present here the mechanism, clinical potential, and a roadmap to clinical trials for this novel approach to control or cure many human cancers.

### Relevant basic immunology

The immune system uses T lymphocytes to identify and control malignant or viral transformation in all cells of the body. CTL recognizing non-self, peptide epitopes expressed on MHC class I molecules, can kill the transformed cell. The surveillance of self-peptides originates with proteosome digestion of cytoplasmic proteins into peptides, which are transported into the ER by the transporter of antigenic peptides (TAP). That repertoire of self-peptides becomes bound to MHC class I molecules in the ER, at the time of their synthesis, and transported to the cell surface for recognition by CD8\(^+\) CTL. MHC class I molecules are expressed on all nucleated cells of the body.

CD4\(^+\) immunoregulatory T helper cells recognize antigenic peptides presented by MHC class II molecules on professional antigen presenting cells (APC), e.g., dendritic cells (DC), macrophages, and B lymphocytes. In such APC, MHC class II molecules do not normally bind the ambient peptides of the ER at the time of their synthesis, because the antigenic peptide binding site of MHC class II molecules is blocked by the Ii protein. The trimer consisting of Ii protein, and MHC class II alpha and beta chains is transported to a post-Golgi antigenic peptide charging compartment where proteases,

---

**Figure 1** Summary of mechanism. Transduction of genes for either MHC class II transactivator (CIITA) or interferon-\(\gamma\) induces tumor cells to express MHC class II molecules and the antigenic peptide binding site binding protein Ii. Co-transduction of a reverse gene construct for a segment of Ii induces an antisense mRNA (X) which blocks transcription of Ii protein. Without the Ii protein, MHC class II molecules in the ER bind a large repertoire of endogenous immunogenic peptides including cryptic epitopes. In Ii-suppressed tumor cells, MHC class I presentation is not interrupted by Ii suppression and thus such tumor cells present simultaneously through both MHC class I (not shown in the figure) and class II molecules to CD8\(^+\) and CD4\(^+\) T cells, respectively. Such simultaneous presentation greatly enhances the activation of CTL. Among the large repertoire of MHC class II-presented peptides are additional ones to be presented by many MHC class II alleles, plus novel epitopes which were previously not dominantly presented, and are therefore candidates for breaking immunosuppression to the cancer.
which also digest internalized antigenic proteins, cleave and release Ii protein in a concerted process of Ii fragments release/antigenic peptide charging. Normally, only exogenous antigens, that are selected for internalization by the APC, are processed for MHC class II presentation. In the case of DC, recognition of MHC class II epitopes activates CD4+ T helper cells. Activated CD4+ T helper cells, in response to that epitope, leads to maturation (licensing) of the DC to stimulate CTL recognizing MHC class I-presented peptides. Likewise, B lymphocytes recognizing MHC class II epitopes processed from antigens internalized by B cell surface immunoglobulins, activate the B lymphocytes to proliferate and mature into antibody secreting plasma cells.

Suppressing expression of the Ii protein by antisense methods leads to MHC class II molecules picking up peptides from the repertoire transported into the ER (Figure 1). Such tumor cells then present tumor antigens to both CD4+ and CD8+ T cells. Activated CD4+ T cells, which are specific for endogenous tumor antigens, contribute to in situ licensing of tumor cells (tumor cell APC). The licensing process involves the in vivo induction of B7 through MHC class II molecules. Transfection of tumor cells with cytoplasmic region deleted MHC class II lost the capability to induce the expression of B7 in vivo. CIITA is a master transcription factor that induces the expression of MHC class II molecules in all tumor lines we have tested. Sal 1 sarcoma line,1 MC-38 colon adenocarcinoma3 and Renca renal adenocarcinoma3 retained good responses to IFN-γ, with MHC class II molecules being induced to same extent as to that induced by CIITA. However, in RM-9 prostate carcinoma cells CIITA must be used to induce the expression of MHC class II molecules. RM-9 cells may be defective in the CIITA gene and IFN-γ cannot induce MHC class II molecules. Nevertheless, in our studies, IFN-γ is required for optimal protection in the RM-9 murine prostate carcinoma model in addition to CIITA to induce MHC Class II molecules. IFN-γ induces MHC class I molecules on the transfected RM-9 tumor cells, consistent with the hypothesis that tumor cells are converted into APC-surrogates to activate CTL.

Pioneer work exploiting use of MHC class II-positive/Ii-negative phenotype in tumor immunotherapy

The concepts underlying our work were identified first by Dr. Suzanne Ostrand-Rosenberg and colleagues. They demonstrated that transfecting syngeneic genes for MHC class II alpha and beta chains into a MHC class II-negative tumor, creates a tumor cell vaccine, which protects against challenge with the parental tumor. In the murine Sal sarcoma model, the parental tumor is MHC class I-positive, but MHC class II-negative. In mice vaccinated with the gene-engineered MHC class II-positive cells, both CD4+ T helper cells and CD8+ CTLs were essential for protection against challenge by parental cells, because antibody-mediated deletion of either cell population, destroyed the protective response.

Supra-transfecting the potent, engineered MHC class II-positive tumor cells with a gene for the Ii protein, abrogated the vaccine potential of the modified cells. That is, the engineered cells were no more potent as vaccine cells than were the parental cells. In the potent vaccine MHC Class II+/Ii-suppressed cells, MHC class II molecules, not blocked by the Ii protein at the time of their synthesis, picked up ambient peptides (including tumor peptides) in the ER. Introducing expression of the Ii protein into such cells again, blocked binding of the ER peptides and destroyed the immunogenicity of the tumor cells, even if MHC class I presentation continued. In these experiments, the T helper cells activate and expand the population of CD8+ CTL. This enhancement of the CTL response seems to be mediated by the “licensing” activity of CD4+ T cells on tumor cells to become APC here, which in turn activate CD8+ T cells more potently.

An additional function of T helper cells, activated by MHC class II-positive/Ii-suppressed tumor cells, has been to prolong memory and protecting mice against tumor challenge for long periods of time after vaccination.

This group further demonstrated that endogenous proteins from many intracellular compartments of a tumor cell can become presented by the MHC class II-positive/Ii-suppressed tumor cells. The gene for hen egg lysozyme (HEL) was engineered with leader sequences targeting to the ER. MHC class II epitopes of HEL were presented to HEL-specific CD4+ T cells when transfected into cells, which were MHC class II-positive but Ii-negative. Co-expression of Ii protein in such cells inhibited presentation of the HEL epitopes. Absence of H-2M, another regulator of antigenic peptide charging to MHC class II molecules, had no effect on endogenous tumor antigen presentation in this model.

Clinically practical tools and methods

In a clinical setting, it is not feasible to transfect a patient’s cells with autologous MHC class II genes
because MHC class II alleles are highly polymorphic. We have used genetic tools to induce endogenous MHC class II molecules (with CIITA or IFN-γ) and suppress li protein (by antisense methods, oligonucleotides or reverse gene constructs).1-4 Tumor cells treated with an active li antisense oligonucleotide were potent vaccine cells.1 However, since antisense oligonucleotides have limited use in vivo, we created expressible li antisense gene constructs. Since there is only one human li allele, our reagents are therefore suitable for use in all patients regardless of MHC class II allele polymorphism.

**Design and in vitro testing of gene constructs**

We first synthesized li antisense oligonucleotides to suppress li expression in MHC class II+/li+ tumor cells.1 In the sarcoma cell (Sal1) tumor model, tumor cells treated with this li antisense oligonucleotide are potent vaccine against challenge by parental tumor. In order to develop clinically useful in vivo therapeutic antisense reagents, we also created expressible li antisense reverse gene constructs (li-RGC). These were constructed by cloning different li gene fragments in reverse orientation into expressible plasmids or adenoviruses, to evaluate multiple methods of tumor cell administration.3,4 The li-RGC genes were evaluated by stable or transient DNA transfections in several murine tumor cell lines, including A20 lymphoma cells, MC-38 colon adenocarcinoma cells, Renca renal adenocarcinoma cells, B16 melanoma cells, and RM-9 prostate cancer cells. The most active one li-RGC (−92,97) (A in the AUG start codon is position 1) was chosen for in vivo studies.

Among the cell lines tested, A20 is already MHC class II+/li+ tumor cells. li-RGC (−92,97) significantly inhibited li expression when this construct was delivered by lipid or gene gun transfection methods. The other tumor lines tested are MHC class II−/li−. These cell lines were co-transfected in vitro with li-RGC (−92,97) and either CIITA or IFN-γ, or both, creating the MHC class II-positive/li-suppressed phenotype.2,4 In vivo induction of the MHC class II-positive/li-suppressed phenotype was also generated by intratumoral injection of li-RGC and CIITA plasmids with lipid2,4 or recombinant adenoviral vectors containing li-RGC (−92,97), CIITA and IFN-γ.3 In summary, we have generated therapeutic MHC class II-positive/li-suppressed phenotype induction constructs, which are biologically very active in all tested cell lines.

**Efficacy in “tumor cure” models**

The in vivo activities of these therapeutic constructs were tested by intratumoral injection in established subcutaneous tumors using two tumor models: the Renca renal carcinoma and the RM-9 prostate carcinoma. In both tumor models, complete regression of established tumors was achieved. In the Renca model, tumor regression was observed in about 50% of mice following four intratumoral injections of CIITA and li-RGC plasmid constructs over four days given together with a suboptimal dose of IL-2 plasmid.2 Intratumoral injections of recombinant adenovirus, containing CIITA, IFN-γ, li-RGC constructs and IL-2 gene, in established Renca tumors induced complete tumor regression in about 60−70% of mice and protection against Renca tumor rechallenge.3 In an aggressive, poorly immunogenic RM-9 prostate tumor model, radiation augmented the effect of the suboptimal dose of IL-2 and MHC class II-positive/li-suppressed phenotype causing complete tumor regression in 50% of the mice.4 Established RM-9 subcutaneous tumors were selectively irradiated and treated a day later with intratumoral plasmid gene therapy using the plasmids pCIITA, pIFN-γ, pIL-2 and pli-RGC for four consecutive days. Data presented in Table 1 showed that intratumoral treatment with all the four plasmids induced complete tumor regression in more than 50% of the mice only when tumor irradiation was administered one day prior to gene therapy. Mice rendered tumor-free by radiation and intratumoral gene therapy and re-challenged on day 64, were protected against RM-9 challenge but not against syngeneic EL-4 challenge (Table 1). These findings demonstrate that in the RM-9 model, radiation enhanced the therapeutic efficacy of intratumoral gene therapy for in situ induction of tumor-specific immune response.

Established RM-9 tumors of 0.3−0.4 cm were irradiated with 8 Gy photons on day 6 after cell injection. From day 7, tumors were injected with the plasmids pCIITA, pIFN-γ, pIL-2 and pli-RGC for four consecutive days. The proportion of tumor-free mice at the end of the observation period, on day 64, following radiation and plasmid therapy, is presented. Tumor-free mice and naive mice were re-challenged with RM-9 cells or unrelated EL-4 cells on day 64, the proportion of challenge-tumor free mice by day 30 is reported.

Reproduced from Hillman et al., Human Gene Therapy 2003; 14: 763−775.4

In order to obtain optimal therapeutic effect, MHC class II and li must be induced with CIITA and li needs to be inhibited by li-RGC in both the Renca
and RM-9 tumor models.²⁻⁴ Our results are consistent with those of Martin et al.¹⁶ who showed, in a murine lung carcinoma model, that induction of MHC class II by CIITA did not create an efficient tumor cell vaccine. This study confirms our finding that induction of MHC class II by transfecting CIITA, which also induces Ii, is insufficient for a therapeutic effect. One must obtain the therapeutic phenotype of MHC class II+/Ii⁻ by also suppressing Ii protein. In order to test for optimal suppression of Ii protein, our therapeutic constructs CIITA and Ii-RGC were used at different ratios. At least a 1:4 ratio (CIITA:Ii-RGC) was required to ensure good inhibition of Ii. IFN-γ is used in the RM-9 prostate tumor to induce MHC class I molecules which are not expressed in the parental cells. Renca cells are MHC class I-positive cells and IFN-γ is not needed to induce MHC class I molecules but does up-regulate further their expression. In both tumor models, a subtherapeutic dose of IL-2 plasmid is needed to promote the immune response.

Given this clear demonstration of efficacy in curing established tumors in mice, and steady progression in preclinical studies to determine optimal treatment protocols, we have begun to develop reagents for treating human cancers. The CIITA gene we used in the mice studies is human and its product functions well on the murine promoters for MHC class II and Ii genes.¹⁷ We also made several human Ii-RGCs, which inhibited Ii expression in a human B lymphoblastoid and the HeLa cell lines. Figure 2 presents the human Ii-RGC (hIi-RGC) induced inhibition of Ii expression in HeLa cells. Transduction of cells with CIITA construct induced up-regulation of cell surface MHC class II molecules and intracellular Ii while transduction of cells with both CIITA and hIi-RGC caused suppression of Ii without affecting enhanced expression of MHC class II. These data were reproduced in additional human tumor cell lines including the human B lymphoma cell line Raji, and human melanoma cell line. We now have in hand the Ii-RGC reagents needed for clinical trials, including human IFN-γ in an expression plasmid.

### Continuing questions in preclinical development

What roles do cytokines play?

In all intratumoral studies a low dose of IL-2 was needed for optimal therapeutic effect. Induction of MHC class II-positive/Ii-suppressed phenotype by treatment with CIITA and Ii-RGC constructs only without IL-2 construct was not sufficient to induce a complete tumor regression, consistent with the observation that injecting only CIITA and Ii-RGC vectors does not elicit an appreciable T cell infiltration into the tumor site.² Addition of an IL-2 gene plasmid provided for local release of IL-2 to promote T cell infiltration and activation.¹⁸ Intratumoral IL-2 plasmid therapy alone, at higher doses

### Table 1 Anti-tumor response of RM-9 tumor-bearing mice treated with radiation and plasmid gene therapy, and response of cured animals to re-challenge

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Post treatment RM-9</th>
<th>Post challenge tumor EL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/10</td>
<td>–</td>
</tr>
<tr>
<td>PCIITA + pIFN-γ + pil-2 + pil-RGC</td>
<td>0/7</td>
<td>–</td>
</tr>
<tr>
<td>Radiation</td>
<td>0/11</td>
<td>–</td>
</tr>
<tr>
<td>Radiation + empty plasmid</td>
<td>0/5</td>
<td>–</td>
</tr>
<tr>
<td>Radiation + pCIITA + pilIFN-γ + pil-2 + pil-RGC</td>
<td>7/13</td>
<td>7/7</td>
</tr>
<tr>
<td>Radiation + pCIITA + pilIFN-γ + pil-2 + pil-RGC</td>
<td>3/6</td>
<td>–</td>
</tr>
<tr>
<td>Naïve mice</td>
<td>NA</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Figure 2 Reagents for treatment of human tumors. Human cervical carcinoma HeLa cells were transduced with MHC class II transactivator (CIITA) or both CIITA and Ii reverse gene construct (hIi-RGC). The cells were immuno-stained for cell surface MHC class II molecules (MHC II) or intracellular Ii protein (Ii) and analyzed by flow cytometry. The human Ii-RGC suppressed Ii protein without affecting MHC class II expression.

In situ tumor autovaccine
of 50 μg/injection, in Renca tumors and ovarian tumors was tumoricidal. However, in our studies, using a subtherapeutic low dose of 2 μg/injection of IL-2 plasmid together with in vivo induction of the MHC class II-positive/Ii-suppressed phenotype effectively shrank or greatly reduced the rate of progression of established Renca tumors. These findings were confirmed in our Renca studies using the same approach with adenoviral vectors. The addition of the IL-2 gene enhanced the response probably by acting as an adjuvant cytokine, helping to strengthen and sustain the activation of both CD4+ and CD8+ T cells. Our data indicate that CIITA plus Ii-RGC alone displays endogenous tumor antigen by both MHC class I and II to initiate antitumor immune response. Induction of a full antitumor immune response requires promotion of T cell proliferation. In RM-9 cells that are MHC class I-negative, addition of IFN-γ gene to CIITA and Ii-RGC genes was required for optimal therapeutic effect. IFN-γ induces expression of MHC class I molecules and also enhances the maturation of DC and NK cells. Our early study showed that IFN-γ plus Ii antisense oligonucleotide inhibition in Sal1 cells offered a more potent antitumor protection than CIITA plus Ii antisense oligonucleotide. IFN-γ is not required to induce a potent therapeutic effect in Renca model since Renca cells are MHC class I-positive. Whether MHC class II-positive/Ii-suppressed phenotype immunotherapy might be used with other cytokines is an open question. Theoretically, CIITA plus Ii-RGC should synergize with other methods to enhance the immune response, such as B7 gene injection.

How does radiation enhance Ii suppression immunotherapy in the prostate tumor model?

In the RM-9 prostate tumor model, a single dose of 8 Gy photon radiation, selectively administered to the tumor prior to gene therapy, enhanced the therapeutic effect of the MHC class II-positive/Ii-suppressed phenotype. Complete tumor regression associated with systemic tumor immunity occurred only when established tumors were first irradiated followed a day later by initiation of intratumoral CIITA, IFN-γ, Ii-RGC and IL-2 plasmid therapy for four consecutive days. Possible mechanisms for radiation enhancement of gene therapy include the following: (1) The debulking effect slows tumor growth so that immunotherapy has time to develop. (2) Radiation-induced tissue damage mobilizes inflammatory cells in the tumor vicinity. (3) Radiation limits suppressive immunoregulatory T cells.

(4) Radiation increases gene transduction efficiency and duration of expression of surviving tumor cells thus increases efficiency of in situ gene modification leading to immune response.

What additional preclinical studies are needed?

Additional pre-clinical studies before initiating a clinical trial include the following: (1) Toxicology and pharmacokinetics of the DNA plasmids or adenoviral vectors will be evaluated, including biodistribution and existing duration of the therapeutic vectors in different tissues and organs. (2) Therapeutic constructs will be optimized, for example by constructing a plasmid that contains both CIITA and multiple copies of Ii-RGC in order to increase the efficiency of inducing the MHC class II-positive/Ii-suppressed phenotype and to decrease the amount of the DNA vectors. (3) The injection schedule/doses of cytokines and frequency of radiation will be optimized. The ratio of injected DNA versus tumor volume needs to be determined. All of these studies are underway. A final concern is to monitor for possible induction of autoimmunity, which might be of therapeutic benefit when restricted to the tumor tissue.

How does this method compare to other in situ therapies?

Several effective therapies have been developed for in situ, as opposed to systemic, treatment of tumors. Such modalities including cryotherapy, external beam radiation, radiation seed implantation (brachytherapy), and photodynamic therapy (porfimer sodium), are approved for patients with prostate cancer, head and neck cancer, and lung cancer. Local injection of approved systemic chemotherapeutic agents is being investigated in addition to the intratumoral injection of the following experimental products: oncolytic viruses, suicide genes, tumor-suppressor genes, and cytokine genes, genes for immunomodulating molecules including B7 and DC.

Most of these approaches kill the affected tumor cells, but do not eradicate distant tumors. The most promising approaches are those that are designed not only to kill the cells that are directly contacted by the intratumoral therapy, but also, that elicit an immune response which in turn eradicates tumor cells and deposits at both locoregional and distant sites. Intratumoral injections of cytokines such as IL-2, TNF-α, also offered substantial therapeutic
effects and elicited tumor-specific immune response. However, turning live tumor cells in situ into CD4+ and CD8+ T cell-stimulating cells is novel and a potent method to induce the widest spectrum of tumor antigen-specific immune responses, and more importantly, this method is synergistic with most other methods mentioned above for an improved therapeutic effect.

Is there an autoimmune response against normal tissues?

Most tumor immunotherapies or vaccines, such as tumor cell-based tumor vaccine and heat shock protein tumor vaccine, face a recurring question. Is there an autoimmune response being induced against normal tissues? In the course of the response to our immunotherapy, as well as other immunotherapies, some functionally important, self-antigens might be exposed to the immune system. We have not had evidence for autoimmune responses in immunopathological studies of 15 organs from surviving mice receiving either antisense oligonucleotides or Ii-RGC treatment (unpublished observation). Furthermore, Ii knockout mice did not have evidence of autoimmune disease.38 Two possible explanations might account for this phenomenon: (1) Tumor antigens are usually abundant or mutated and thus are of much stronger immunogenicity while normal antigens are tolerated during development and are of much weaker immunogenicity. (2) Autoimmunity induction is organ- or tissue-specific. In the Sal1 sarcoma model, no autoimmunity was induced.1 However, this finding does not mean that no autoimmunity is induced in other tumor model. For example, in Hashimoto’s thyroiditis, we have observed discordant expression of MHC class II and Ii, suggesting MHC class II+/Ii- thyocytes may present endogenous antigens to induce thyroiditis.39 Finally, one must consider the possibility that a local autoimmune response within the injected tumor probably contributes to the tumoricidal effect.

Are there other uses of MHC class II-positive/Ii-suppressed phenotype-inducing constructs?

Other potential uses of MHC class II-positive/Ii-suppressed phenotype induction include defining tumor antigens and enhancing DNA vaccines. Tumor-related antigenic epitopes can be identified by tandem HPLC mass spectrometry of acid-eluted peptides from immunopurified MHC class II molecules. Eluted-peptide HPLC patterns can be compared with those of MHC class II-positive/Ii-positive cells to identify putative Ii suppression-specific peaks. The molecular weight of a peptide in such a peak can be precisely determined by tandem mass spectrometry and a sequence imputed from the weight. An alternative method to identify tumor antigens, which are preferentially presented in mice with Ii-suppression treated tumors, is to develop T cell lines. Those lines can be used as indicators for those antigens in further studies with progressive fractions of tumor cell lysates. By progressive fractionation of stimulating fractions, often a specific tumor antigen gene is identified.

A second use is to enhance a DNA vaccine. The biological effect of Ii suppression will enhance the immune response to a co-delivered DNA vaccine for a malignant or infectious antigen. When administered into the skin by gene gun impelling of DNAs adsorbed to gold particles, a strong response is registered.

Clinical trials

Clinical trials of our intratumoral gene therapy will evaluate safety and efficacy. Phase I/II and II studies can be carried out in patients with accessible cancers (e.g., breast, colorectal and prostate cancers, or melanoma), however, Phase III trials will be restricted to a selected type of cancer. Intratumoral gene therapy to induce MHC class II molecules and suppress Ii protein is administered in multiple injections (e.g., once a day for 2–4 days) into one or more tumor nodules in a patient. Safety is the first issue. We will examine and test for local toxicity (pain, inflammation, and other locoregional site reactions) and systemic toxicity (hematological, hepatic or renal toxicity) and autoimmunity. Pharmacokinetics (blood and urine) will be analyzed to evaluate the biodistribution of therapeutic plasmids. The antitumor immune response will be evaluated by tumor-specific ELISPOT assays. In vivo induction of the therapeutic phenotype and CD4+ and CD8+ lymphocyte infiltration will be measured in early biopsies.

A focus of Phase II trials is evaluating regression in patients with refractory tumors, at both injected and distant sites. The ultimate planned optimal use of our active immunotherapy provides for consolidation therapy. However, before undertaking trials in patients induced to no evidence of disease (NED) status (usually by surgery and adjuvant chemotherapy, but with suspected micrometastatic disease), efficacy of our protocol must be demonstrated in
measurable lesions. There are two complicating issues. First, patients in Phase II trials with refractory disease will concurrently be undergoing chemotherapy, which suppresses the immune response. Secondly, measurable smaller lesions need to be present since active immunotherapy does not work well on large masses. Myelosuppression secondary to adjuvant chemotherapy can be assessed by PBMC and BM analyses.

Additional studies will also be performed in newly diagnosed patients with no clinical evidence of disease outside the resection margins. This is an ideal setting for active immunotherapy; however, much longer-term follow-up of patients is required to determine therapeutic benefit because time to progression and survival are greatly enhanced in this group of patients compared to those undergoing surgery and adjuvant chemotherapy for advanced disease.

A first clinical trial might well target colorectal adenocarcinoma for the following reasons: (1) Tumor masses are readily accessible with minimally invasive techniques including sigmoidoscopy and colonoscopy. (2) Surgery or radiation therapy + combination chemotherapy is routinely administered. (3) Accessible recurrent tumors requiring resection and second-line chemotherapy often occur. (4) Evaluation of patients for recurrence and progression are routine.

Conclusions

One advantage of intratumoral induction of the MHC class II-positive/Ii-suppressed phenotype is that prior identification of tumor antigens is not necessary. Ii “unblocked” MHC class II molecules survey the antigenic peptide pool in ER and present whatever tumor epitopes are bound in the ER to activated CD4+ T cells. This provides a better chance to prevent the tumor from escaping the host’s immune surveillance since a broader spectrum of heterogeneous tumor antigens is expected to be surveyed by MHC class II molecules. CIITA also enhances MHC class I expression, especially when class I expression is diminished and thus class I antigen presentation is also promoted. This effect is significant because deletion of MHC class I alleles is frequently a way for tumors to escape immunosurveillance. Another advantage of MHC class II-positive/Ii-suppressed phenotype induction is that it can be synergistic with other antitumor therapies such as injections with IL-12 and B7 genes. A final advantage is that the monomorphic structure of the Ii gene means that one vector construct can be used in all patients, regardless of the heterogeneity of MHC class II alleles.

Conversion of cancer cells into APC via induction of the MHC class II-positive/Ii-suppressed phenotype in vivo by this method is simple to achieve. Transduction of a focal population of cells within a tumor mass stimulates an immune response that potentially leads to destruction of all cells within the mass as well as in metastases. This is particularly relevant to the anticipated clinical use where local treatment to induce a potent systemic antitumor response is the goal.

Intratumoral II-RGC therapy offers great potential in the treatment of patients with solid malignancies. The goal of therapy is “immuno-consolidation”, that is, to induce a potent antitumor immune response capable of eradicating tumor cells throughout the body that are left behind following surgery or radiation, or are not killed by adjuvant chemotherapy. The concept is presented in Table 2: (1) some cells within a tumor mass are converted to APC that elicit a robust CD4+ and CD8+ T cell immune response; (2) the effector cells recognize cancer cells within the primary tumor that were not transduced as well as cells near the primary tumor and at distant sites; (3) upon recognition by the immune system, the residual cancer cells are destroyed. The development and progression of cancer is associated with the inability of the immune system to recognize rogue cells as aberrant and to attack and kill the cells. Therefore, uncloaking the tumor cells via transduction of the MHC class II-positive/Ii-suppressed phenotype, thereby enabling induction of a potent CD4+ and CD8+ T cell antitumor immune

Table 2 Main points

- Intratumoral gene therapy to induce an antitumor immune response is feasible.
- Novel patient-specific vaccine cells are created in vivo with a therapeutic phenotype of MHC class II-positive/Ii-suppressed.
- A small number of transduced cells elicit a robust CD4+ T helper cell-directed, immune response against all cancer cells – locally and at distant sites. These responses lead to enhanced progression-free and overall survival.
- Active immunotherapy works best in small volume disease states.
- Active immunotherapy is a valuable adjunct to surgery and radiation for immuno-consolidation.
response, should greatly improve clinical outcomes in patients with cancer.

References

1. Qiu G, Goodchild J, Humphreys RE, Xu M. Cancer immuno-
therapy by antisense suppression of II protein in MHC-class
II-positive tumor cells. Cancer Immunol Immunother

2. Lu X, Kalilintser NL, Li J, et al. Tumor immunotherapy by
converting tumor cells to MHC class II-positive, II protein-
negative phenotype. Cancer Immunol Immunother

II+/II− phenotype after adenoviral delivery of both an
expressible gene for MHC class II inducer and an antisense

intratumoral gene therapy for induction of cancer vaccine in
murine prostate carcinoma. Human Gene Ther
2003;14:763–75.

5. Bryant PW, Lennon-Dumenil AM, Fiebiger E, Lagaudriere-
Gesbert C, Ploegh HL. Proteolysis and antigen presentation

6. Daibata M, Xu M, Humphreys RE, Reyes VE. More efficient
peptide binding to MHC class II molecules during cathepsin B
digestion of II than after II release. Mol Immunol

7. Xu M, Capraro GA, Daibata M, Reyes VE, Humphreys RE.
Cathepsin B cleavage and release of invariant chain from
MHC class II molecules follow a staged pattern. Mol Immunol

8. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic
cell can be a temporal bridge between a CD4+ T-helper and

9. Baskar S, Clements VK, Gilmer LH, Nabavi N, Ostrand-
Rosenberg S. Rejection of MHC class II-transfected tumor
cells requires induction of tumor-encoded B7-1 and/or B7-2

10. Clements VK, Baskar S, Armstrong TD, Ostrand-Rosenberg S.
Invariant chain alters the malignant phenotype of MHC class

11. Baskar S, Azarenko V, Garcia Marshall E, Hughes E, Ostrand-
Rosenberg S. MHC class II-transfected tumor cells induce
long-term tumor-specific immunity in autologous mice. Cell

12. Baskar S, Gilmer L, Nabavi N, Jones RT, Ostrand-Rosen-
berg S. Major histocompatibility complex class II+B7-1+
tumor cells are potent vaccines for stimulating tumor

13. Armstrong TD, Clements VK, Martin BK, Ting JP, 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

14. Qi L, Ostrand-Rosenberg S. MHC class II presentation of
endogenous tumor antigen by cellular vaccines depends on
the endocytic pathway but not H2-M. Traffic

15. Qi L, Rojas JM, Ostrand-Rosenberg S. Tumor cells present
MHC class II restricted nuclear and mitochondrial antigens
and are the predominant antigen presenting cells in vivo. J

16. Martin BK, Frelinger JG, Ting JP. Combination gene therapy
with CD86 and the MHC class II transactivator in the control

17. Ting JP, Trowsdale J. Genetic control of MHC class II

18. Horton HM, Dorigo O, Hernandez P, Anderson D, Berek JS,
Parker SE. IL-2 plasmid therapy of murine ovarian carcinoma
inhibits the growth of tumor ascites and alters its cytokine

19. Saffran DC, Horton HM, Yankukas MA, et al. Immunother-
apy of established tumors in mice by intratumoral injection
of interleukin-2 plasmid DNA: induction of CD8+ T-cell

renal carcinoma pulmonary metastases by systemic admin-
istration of interferon gamma: Mechanism of action and
potential for combination with IL-4. Clin Cancer Res

21. Stevens CW, Zeng M, Cerniglia GJ. Ionizing radiation greatly
improves gene transfer efficiency in mammalian cells. Hum

continuously infused intratumoral bleomycin for the treat-
ment of recurrent glioblastoma multiforme. J Neurooncol
2002;60:37–42.

23. Harbord M, Dawes RF, Barr H, et al. Palliation of patients
with dysphagia due to advanced esophageal cancer by
dendritic cell vaccination with recombinant vaccinia virus
expressing the Epstein-Barr virus-encoded lytic protein

intralesional immunotherapy for metastatic melanoma. Cancer

25. Miyagi T, Koshida K, Hori O, Konaka H, Katoh H, Kitagawa Y,
et al. Gene therapy for prostate cancer using the cytosine
deaminase/uracil phosphoribosyltransferase suicide system.

26. Teh BS, Aguilar-Cordova E, Kernen K, et al. Phase I/II trial
evaluating combined radiotherapy and in situ gene therapy
with or without hormonal therapy in the treatment of
prostate cancer: a preliminary report. Int J Radiation Oncol

27. Swisher SG, Roth JA, Komaki R, et al. Induction of p53-
regulated genes and tumor regression in lung cancer patients
after intratumoral delivery of adenoviral p53 (INGN

immune responses to adenosine and p53 protein antigens in
patients following intratumoral injection of an adenosine
vector expressing wild-type. P53 (Ad-p53). Cancer Gene

tumoral recombinant GM-CSF-encoding virus as gene ther-
apy in patients with cutaneous melanoma. Cancer Gene

30. Fuji S, Huang S, Song TC, et al. Induction of melanoma-
associated antigen systemic immunity upon intratumoral
delivery of interferon-gamma retroviral vector in melanoma

therapy for prostate cancer: phase I clinical trial and basic

32. Rochlitz C, Dreno B, Jantscheff P, et al. Immunotherapy of
metastatic melanoma by intratumoral injections of Vero
cells producing human IL-2: phase II randomized study
comparing two dose levels. Cancer Gene Ther 2002;9(3):
289–95.


