Review

Forcing Tumor Cells to Present Their Own Tumor Antigens to the Immune System: a Necessary Design for an Efficient Tumor Immunotherapy

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The general principle for tumor cells to escape from immune surveillance is to prevent tumor antigens from being recognized by the immune system. Many methods have been developed to increase the immunogenecity of the tumor cells. The most efficient methods are able to force tumor cells to present their own tumor antigens to the immune system. Stimulating Th cells by converting tumor cells into MHC class II+/Ii- antigen presenting cells is one of the most efficient technologies. Using antisense methods, we suppress the expression of the Ii protein that normally co-expresses with MHC class II molecules and blocks the antigenic peptide binding site of MHC class II molecules during synthesis in the endoplasmic reticulum. In such tumor cells, the "unprotected" MHC class II molecules pick up endogenous tumor antigenic peptides, which have been transported into the ER for binding to MHC class I molecules. Simultaneous presentation of tumor antigens by both MHC class I and II molecules generates a robust and long-lasting anti-tumor immune response. MHC class II+/Ii- tumor cells are potent tumor cell vaccines and also cure 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. *Cellular & Molecular Immunology*. 2004;1(3):180-185.

Key Words: immunotherapy, cancer vaccine, MHC class II, Th cell

Introduction

Tumor immunotherapy has been rapidly developed into a promising therapy for tumors. The tumor vaccines include DNA vaccines, peptide vaccines, and tumor cell-based vaccines or immunotherapy. DNA and peptide vaccines need the prior identification of tumor related antigens. However, tumor cell-based immunotherapy does not need to define tumor antigens in advance. The major modifications of tumor cells have been used to increase the immunogenicity of tumor cells. The common methods are genetically engineering the tumor cells to express cytokines (1, 2). Other methods include dendritic cells (DCs) pulsed with tumor cell lysate or tumor antigen DNA (3, 4) and tumor cell/DC fusion cells (5, 6). Investigators also use chemicals to modify tumor cells to increase their immunogenicity (7). However, many methods such as IL-2 and GM-CSF therapies (8, 9) that have been used to increase the immunogenicity only non-specifically activate the immune system. A most efficient method should be able to force tumor cells to actively present their own tumor antigens to the immune system. These methods include IFN-γ modified tumor cells and tumor cell/DC fusion cells.

While IFN-γ increases the expression of MHC class I molecules on some tumor cells, it does not generate a robust immune response against tumor cells. One of the reason is that MHC class I presentation alone is not sufficient to activate $CD8^+$ T cells without the help from tumor antigen-specific $CD4^+$ Th cells. MHC class II presentation has thus been getting more attention in tumor immunotherapy design (10-12). However, induction of MHC class II on tumor cells by Class II transactivator (CIITA) again can not confer an efficient tumor cell-based immunotherapy because the co-induction of Ii protein with MHC class II by CIITA (13) prevents the presentation of endogenous tumor antigens from MHC class II molecules. We have developed a technology that generates MHC class II+/Ii- phenotype tumor cells and it is potent tumor cell immunotherapy.

Relevant basic immunology

The immune system uses T lymphocytes to identify and

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Abbreviations: ER, endoplasmic reticulum; CIITA, Class II transactivator; DC, dendritic cell; TIL, tumor infiltrating lymphocyte; HEL, hen egg lysozyme; Ii-RGC, Ii reverse gene constructs.

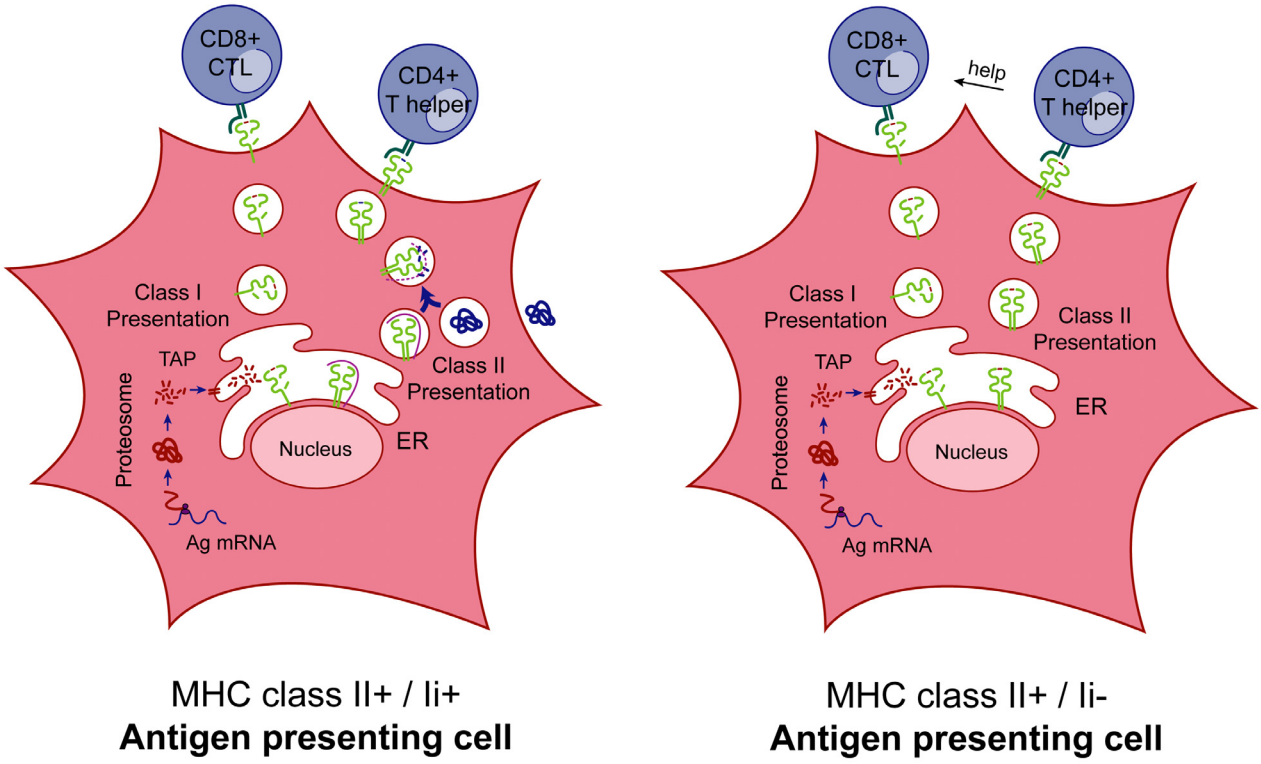

Figure 1. Diagram of mechanisms of MHC class I and class II antigen processing and presentation pathways. Left, MHC class I presents endogenous antigens to $CD8^+$ T cells and MHC class II molecules present exogenous antigen to MHC $CD4^+$ T cells. Ii protein blocks the binding of endogenous antigens to MHC class II in the ER. Proteosome and TAP are also indicated for the processing and transportation of endogenous antigens to the ER. Right, without Ii protein expression, endogenous antigens, including tumor antigens if this is a tumor cell, are also presented by MHC class II molecules. The simultaneous presentation of endogenous tumor antigens by both MHC class I and II molecules generates a potent tumor cell immunotherapy.

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 endoplasmic reticulum (ER) by the TAP (Figure 1). 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. $CD4^+$ Th cells recognize antigenic peptides presented by MHC class II molecules on professional APC. Normally, only exogenous antigens are processed for MHC class II presentation for the activation of $CD4^+$ Th cells. Activated $CD4^+$ Th cells help the full activation and expansion of $CD8^+$ CTL. Without the help from $CD4^+$ Th cells, recognition of MHC class I/peptide complex by $CD8^+$ CTL causes anergy of CTL rather than activation.

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 (Figure 1) (14, 15). In tumor cells, it has been demonstrated that only Ii-negative Sal1 cells present ER-retained antigenic peptides (16-18). It is clear now that Ii protein played an important role in preventing endogenous antigenic peptides in the ER from being presented by MHC class II molecules. In tumor cells, super-induction of Ii protein renders tumor cells unrecognized by $CD4^+$ T cells, leading to the escape from immune surveillance even if MHC class I recognition occurred. Under normal condition, Ii protein expression is always co-regulated with MHC class II molecules. By creating MHC class II+/Ii- phenotype tumor cells, one can let tumor cells present their own tumor antigens by MHC class II to activate $CD4^+$ T cells, producing a potent tumor cell immunotherapy (Figure 1).

Mechanisms for tumors to escape from immune surveillance

Several mechanisms have been proposed whereby tumor cells may remain non-immunogenic (19-21). Deletion of MHC class I alleles or the β_2 microglobulin gene, for example, is a common feature that makes tumor cells incapable of presenting tumor antigens by MHC class I molecules. The deletion of MHC class I is frequently accompanied with the deletion of TAP-1 and TAP-2 proteins that block the pathway for the processed tumor antigenic epitopes into the ER (22-24). Deletion of subunit of proteosome, such as LMP-1, in several tumors blocks the processing of tumor antigens (25). Deletion of the H2-DM gene ensures that MHC class II molecules on

tumor cells remain continuously occupied with CLIP; cells with this phenotype are incapable of presenting even exogenous antigens (26). Many normal tissues do not express MHC class II molecules and the Ii protein. However, the induction of MHC class II and Ii genes occurs in many types of tumors (27-31). In all tumors examined, a common finding is that Ii protein is always superinduced relative to the induction of MHC class II molecules which were often deleted again thereafter. We found that Ii protein induction correlated significantly with malignancy of the tumor and was inversely correlated with the level of tumor infiltrating lymphocytes (TIL) while there were no correlations found between HLA-DR and malignancy and TIL (32). These data suggest that increased expression of Ii protein may prevent the presentation of endogenous tumor antigens by MHC class II molecules and thus confer tumor cells with another mechanism to escape from immune surveillance. Recently, it has been shown that failure of induction of MHC class II and Ii protein might also be a mechanism for tumor cells to escape the immune surveillance. For example, in small cell lung cancer, CIITA mutation causes unresponsiveness of these cells to IFN-γ for the induction of MHC class II and Ii protein (33).

Generation of MHC class II+/Ii- phenotype creates potent tumor cell-based vaccine and immunotherapy

Early work exploring the use of MHC class II+/Ii- phenotype in tumor vaccine

The concepts underlying our work were first identified by the group led by Dr. Ostrand-Rosenberg who demonstrated that transfecting syngeneic genes for MHC class II alpha and beta chains into a MHC class II- tumor, generates a potent tumor cell vaccine, which protects against challenge with the parental tumor (11-13, 34). *In vivo* experiments have shown that the gene-engineered MHC class II+ cells generated an efficient tumor cell vaccine. Supra-transfecting this MHC class II+ tumor cells with a gene for Ii, abrogated the vaccine potential of the modified cells (35). In the vaccine MHC class II+/Ii- 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 and then presented tumor antigens to activate $CD4^+$ T cells. Introducing expression of the Ii protein into such cells, blocked binding of the ER peptides and destroyed the immunogenicity of the tumor cells, even if MHC class I presentation continued.

This group further demonstrated that endogenous proteins of a tumor cell become presented by the MHC class II-/Ii- tumor cells (11-13). 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 cells were MHC class II+ but Ii-. Coexpression of Ii protein in such cells inhibited presentation of the HEL epitopes (36).

Vector reagents for generating MHC class II+/Ii- phenotype

Since the highly polymorphism of MHC class II alleles, transfecting each patient's tumor cells with its own MHC class II genes is not practical in the clinic. We have developed a potent tumor cell immunotherapy for many tumors (37-41). This method uses simple gene-regulating reagents potentially usable in all patients to generate MHC class II+/Ii- phenotype, stimulating $CD4^+$ Th cells, which would upregulate CTL.

We first synthesized Ii antisense oligonucleotides to suppress Ii protein expression in MHC class II+/Ii+ tumor cells (37). In Sal1 tumor model, tumor cells treated with Ii antisense oligonucleotide are potent vaccine against challenge by parental tumor. In order to develop clinically useful antisense reagents, we have created expressible Ii reverse gene constructs (Ii-RGC). These were constructed by cloning different Ii gene fragments in reverse orientation into expressible plasmids or adenoviruses (38, 39). The activity of Ii-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, and RM-9 prostate cancer cells. *In vivo* induction of the MHC class II+/Ii- phenotype was also generated by intratumoral injection of Ii-RGC and CIITA plasmids with lipid (38-41) or recombinant adenoviral vectors containing Ii-RGC (-92, 97), CIITA and/or IFN-γ (39). The most active Ii-RGC (-92, 97) (A in the AUG start codon is position 1) was chosen for *in vivo* studies.

Efficacy in curative tumor models

The *in vivo* activities of these therapeutic constructs were tested by intratumoral injection in established subcutaneous models. In the Renca model, tumor regression was observed in about 50% of mice following four intratumoral injections of CIITA and Ii-RGC plasmid constructs over 4 days given together with a suboptimal dose of IL-2 plasmid (38). Intratumoral injections of recombinant adenovirus, containing CIITA, IFN-γ, Ii-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 (39). In an aggressive, poorly immunogenic RM-9 prostate tumor model, injection of the suboptimal dose of IL-2 and MHC class II+/Ii- phenotype inducers caused complete tumor regression in 50%-70% of the mice when tumors were subject to prior irradiation (40). Mice rendered tumor-free were protected against RM-9 challenge but not against syngeneic EL-4 challenge when they were re-challenged on day 64.

Optimal therapeutic effect was obtained in groups in which Ii protein was suppressed by Ii-RGC in both tumor models (38-41). This finding is consistent with those of Martin et al. (13) who also showed that induction of MHC class II by transfecting CIITA did not produce a potent tumor vaccine. For optimal efficacy, IFN-γ must be 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+ cells and IFN-γ is not needed to induce MHC class I molecules. In both tumor models, a sub-therapeutic dose of IL-2 plasmid is needed to promote the immune response.

We have developed reagents for treating human cancers in clinic (41). 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 (42). We also

made several human Ii-RGCs, which inhibited Ii protein expression in HeLa cell line. Transduction of cells with CIITA construct induced expression of cell surface MHC class II molecules and intracellular Ii protein while transduction of cells with both CIITA and hIi-RGC caused suppression of Ii protein without affecting enhanced expression of MHC class II.

Advantages of MHC class II+/Ii- phenotype tumor immunotherapy

The major advantage of using Ii protein suppression to augment MHC class II presentation of endogenous antigens is that it induces endogenous antigen-specific $CD4^+$ T cell activation, while the induction of $CD8^+$ T cells is not interrupted. Endogenous tumor antigens are also released and phagocytosed by DCs or other APCs, processed through the exogenous pathway and expressed on MHC class II molecules for antigen presentation to Th cells (43). However, the availability of soluble antigen to charge MHC class II molecules is limited by the low levels of antigen released (e.g., by cell death) as well as the rapid diffusion and degradation of antigen. In fact, Sauter et al. (44) have shown that apoptotic cells may be incapable of activating DCs. Our Ii inhibition technology overcomes this ineffective activation of $CD4^+$ T cells by enabling locally concentrated MHC class I and class II antigen processing and presentation by living tumor cells.

Another advantage is that one does not need to define tumor antigen in advance. The use of only one MHC class II allele (such as transfection of syngeneic MHC class II molecules) may limit the epitope repertoire that can be displayed by other MHC class II alleles including HLA-DR, -DP, and -DQ. Given the high heterogeneity of tumor antigens in primary and metastatic tumors, even within the same tumor, it is difficult to know which MHC class II allele can best present which tumor antigen for immune stimulation. For these reasons, we believe that induction of endogenous MHC class II molecules, using CIITA for example, combined with Ii antisense to generate MHC class II+/Ii- phenotype, represents the simplest and most effective way of generating a universal cancer cell vaccine.

Compared with other antisense applications such as for oncogene inhibition (45, 46), the technical advantage is that our Ii antisense method does not need to suppress Ii protein expression in all tumor cells permanently which is unconquerable obstacle for other antisense applications. In contrast, our Ii antisense method only needs to suppress Ii temporarily in portion of tumor cells. Immune system will take over the job to eliminate the residual tumor cells after it has been activated by Ii suppression.

Conversion of cancer cells into antigen presenting cells *via* induction of the MHC class II+/Ii- phenotype *in vivo* by this method is simple to achieve. Ii suppression does not conflict with other methods of vaccine enhancement. Ii suppression technology can be used in combination with all other vaccine methods including: cytokines, co-stimulatory molecules, and adjuvants. The induction of MHC class II molecules and Ii by CIITA and suppression of Ii by Ii-RGC antisense is a clinically practical strategy since both CIITA and Ii genes are monomorphic. Transduction of a focal population of cells within a tumor mass stimulates an immune response that potentially leads to destruction of all tumor cells within that mass as well as in distant metastases. This is particularly relevant to the anticipated clinical use where local treatment to induce a potent systemic anti-tumor immune response is the goal.

Other considerations and utilities of Ii suppression

The induction of autoimmunity to normal self antigens becomes a concern in this immunotherapy. We have not had evidence for autoimmune responses in histopathological studies of 15 organs from surviving mice received antisense oligonucleotides (unpublished observation). Two possible mechanisms might account for this phenomenon: 1) Tumor antigens are usually abundant or mutated and thus are stronger immunogens while normal antigens are tolerated during development process and are much weaker immunogens; 2) Autoimmunity induction is organ- or tissue-specific. In the Sal1 sarcoma model, no autoimmunity was induced (37). 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 protein, suggesting MHC class II+/Ii- thyrocytes may present endogenous antigens to induce thyroiditis (47). Finally, one must consider the possibility that a local autoimmune response within the injected tumor probably contributes to the tumoricidal effect.

Other potential uses of MHC class II+/Ii- phenotype induction include defining tumor antigens, enhancing DNA vaccines, and enhancing the efficiency of tumor cell/DC fusion cells. Tumor antigenic epitopes can be identified by tandem HPLC mass spectrometry of acid-eluted peptides from purified MHC class II molecules. Eluted-peptide HPLC patterns can be compared with those of MHC class II+/Ii+ 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. Another 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. Ii suppression can also be used for tumor cell/DC fusion cells, tumor antigen RNA-pulsed DC to enhance the potency of these vaccines.

Conclusion

Tumor cell-based immunotherapy is currently a most efficient and practical tumor immunotherapy. Enhancement of tumor cell immunogenicity is wildly considered and performed in tumor cell-based immunotherapy. The most effective methodologies, however, should be able to force tumor cells to present their own antigens to immune system. Induction of MHC class I molecules alone on tumor cells is not sufficient to induce an efficient anti-tumor immune response. We have developed a method to generate MHC class II+/Ii- phenotype. 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 $CD4^+$ Th cells without interrupting the presentation of MHC class I presentation. The simultaneous presentation of tumor antigens by both MHC class I and class II generated a robust tumor immunotherapy. Conversion of cancer cells into antigen presenting cells *via* induction of the MHC class II+/Ii- 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. Alternatively, re-infusion of *ex vivo* generated MHC class II+/Ii- tumor cells could be used to prevent the recurrence of tumors after a surgery.

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