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Genetic modulation of tumor antigen presentation

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An effective cancer-cell vaccine is created by expressing major-histocompatibility-complex (MHC) class II molecules without the invariant chain protein (Ii) that normally blocks the antigenic-peptide-binding site of MHC class II molecules at their synthesis in the endoplasmic reticulum. Such tumor-cell constructs are created either by the transfer of genes for MHC class IIα and β chains, or by the induction of MHC class II molecules and Ii protein with a transacting factor, followed by Ii suppression using antisense methods. Preclinical validation of this approach is reviewed with the goal of using this immunotherapy for metastatic human cancers.

Several approaches create effective tumor-cell vaccines for cancer patients. These methods include: genetically engineering tumor cells to express and secrete cytokines, engineering tumor cells to express costimulatory, cell-surface molecules to activate T lymphocytes, and creating DNA vaccines of tumor-associated antigens[1–5]. These methods either stimulate the host's immune system or modify tumor cells to express their tumor antigens directly for immune stimulation. Although early studies in this field focused on the activity of cytotoxic $CD8^+$ T cells, more-recent studies have targeted $CD4^+$ T-helper cells for recruitment in tumor immunotherapy. Indeed, studies using $CD4^+$ T-helper

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Glossary

Antigen presentation Peptides of antigenic proteins are bound into MHC class I or MHC class II molecules for presentation at the cell surface of an antigen-presenting cell to be recognized by receptors on the surface of $CD8^+$ cytotoxic or $CD4^+$ immunoregulatory T lymphocytes.
MHC class I molecules Major-histocompatibility-complex class I molecules that bind antigenic peptides for presentation to cytotoxic T cells ($CD8^+$).
MHC class II molecules Major-histocompatibility-complex class II molecules that bind antigenic peptides between two anti-parallel alpha helixes, on a beta-pleated sheet, for presentation to T-helper cells ($CD4^+$).
Ii protein (invariant chain) Monomorphic type II glycoprotein that binds to the antigenic binding site of MHC class II molecules during synthesis to protect them from binding and presenting endogenous peptides.
Licensing of dendritic cells The process of activating macrophage-lineage dendritic cells by stimulated $CD4^+$ T cells, interacting through cell-to-cell recognition molecules, leading to the enhanced ability of dendritic cells to activate $CD8^+$ cytotoxic T lymphocytes.
T-helper cells The subset of T lymphocytes that recognize antigens presented by MHC class II molecules and orchestrate the cascade of the immune response.
Cytotoxic T cells The subset of T lymphocytes that recognize antigens presented by MHC class I molecules and kill virally infected cells, cancers and grafts.

cells to stimulate T-helper cells with MHC class II presented antigenic epitopes have demonstrated significant anti-tumor activity[6–11].

In several studies, investigators transfected tumor cells with genes for syngeneic MHC class II molecules (i.e. the primary mediators of antigen presentation to $CD4^+$ T-helper cells; see Glossary), to produce effective tumor-cell-based vaccines[8–10,12,13]. Upon attempting to repeat this effect by inducing MHC class II molecules with the MHC class II transactivator (CIITA), the tumor cells were not effective as a vaccine[13,14]. This outcome was ascribed to the co-induction of the immunoregulatory protein Ii in such CIITA-transfected tumor cells.

The normal function of the Ii protein is to block the antigenic-peptide-binding site of MHC class II molecules at the time of their synthesis in the endoplasmic reticulum (ER). Thereby, the MHC class II molecules do not survey the ambient peptides that have been introduced into the ER by the transporter of antigenic peptides (TAP) after cytosolic selection and cleavage by proteosomes. Such ambient peptides are normally bound in the ER to MHC class I molecules, toward immunosurveillance of self epitopes. MHC class II molecules normally receive antigenic peptides from selected foreign proteins in a specialized post-Golgi, antigenic peptide charging compartment. By suppressing Ii protein, the ambient antigenic determinants of the ER become bound to MHC class II molecules and are expressed in the immune system. Taken together, these studies provide a conceptual framework guiding preclinical development of this novel approach to cancer-cell-based vaccines.

The need for practicality in any useful clinical approach to tumor immunotherapy speaks against MHC class II gene transfections that are individualized on a per patient basis. It is more efficient to induce MHC class II molecules with CIITA and to suppress the Ii protein by antisense methods. This review summarizes recent work demonstrating: (1) enhanced tumor immunogenicity by expansion of the MHC class II epitope repertoire; (2) function of the Ii protein in endogenous antigen presentation; (3) possible relevance of high Ii expression in several tumor types; and (4) further progress in the development of tumor-cell-based vaccines resulting from an expansion of the repertoire of MHC class II presented antigens.

Expanded MHC class II antigen repertoire increases the efficacy of tumor-cell vaccines

As indicated, tumor cells transfected with genes encoding syngeneic MHC class II molecules produce an effective cell-based cancer vaccine[8–10,12]. This immunization strategy prevents the growth of subsequently administered parental tumor cells, regresses primary tumors that are already established and inhibits tumor metastasis[8–10,15,16]. Immunotherapy using MHC class II transfected tumor cells is based on the hypothesis that these engineered tumor cells present endogenous tumor antigens in the context of MHC class II molecules to tumor-antigen-specific $CD4^+$ T helpers. Specifically, the expanded repertoire of epitopes presented by MHC class II molecules to $CD4^+$ T helpers in the absence of the Ii protein includes novel endogenous tumor antigens that are otherwise not available for immune stimulation. The $CD4^+$ T-helper cells, in turn, facilitate a long-lasting anti-tumor immune response via activation of tumor-antigen-specific $CD8^+$cytotoxic T cells[8–10,12,13]. Without the help of activated $CD4^+$ T cells, $CD8^+$ T cells are insufficiently activated when stimulated by tumor antigens to cause tumor-cell death. The adoptive transfer of immune $CD4^+$ cells, but not $CD8^+$ cells, is sufficient to educate naive mice against subsequent challenge with parental tumor cells[12].

Studies have shown that to produce an effective tumor-cell-based vaccine, the expression of both MHC class I and II molecules on tumor cells is needed[15,16]. Because MHC alleles are highly polymorphic, however, it is impractical to consider using transfections with MHC genes for clinical immunotherapy studies. To overcome this obstacle, it has been proposed that the CIITA gene can be used to induce endogenous MHC class II (and class I) gene expression[13,14]. However, tumor-cell-based vaccines wherein MHC class II genes have been induced by CIITA fail to elicit an effective immune response against a subsequent challenge by parental tumor cells. Presumably, the reason for this is that CIITA also induces Ii protein expression, which in turn protects the antigenic binding site of MHC class II molecules from charging by endogenously synthesized tumor antigens in the ER. This concept is supported by early studies demonstrating that cotransfection of tumor cells with MHC class II and Ii genes abolishes the efficacy of the resulting tumor-cell vaccine that is obtained using MHC class II genes alone[17]. It has also been shown that tumor cells capable of presenting specific endogenous antigens to $CD4^+$ T cells, as a result of transfection with syngeneic MHC class II molecules, lose that ability when cotransfected with the Ii gene[9,10,13]. In the same system, cells induced for MHC class II expression using the CIITA gene (also induces Ii expression) do not present the endogenous antigen[13].

Novel methods for inhibiting Ii gene expression have been developed using Ii antisense oligonucleotides and reverse-gene constructs in MHC class II$^+$ tumor cells[18]. Further, this has been coupled with the use of interferon-γ or CIITA gene transfection to selectively induce MHC class II expression. Tumor cells induced for MHC class II expression, combined with antisense inhibition of Ii, generate effective protective immunity against a subsequent challenge of parental SaI cells; untreated cells do not provide such immunity[18] (Fig. 1).

In summary, the combined use of Ii antisense and transfection with the CIITA gene produces a cell-based vaccine with similar efficacy to that obtained when tumor cells are transfected with syngeneic MHC class II genes in the absence of Ii gene expression. Because the Ii gene is monomorphic, the use of Ii antisense is applicable to all tumors in every individual. Thus, combined use of the CIITA gene and Ii antisense can be used against a broad spectrum of tumors; this is a simple and clinically feasible method of generating MHC class II$^+$ and Ii$^-$ tumor-cell vaccines.

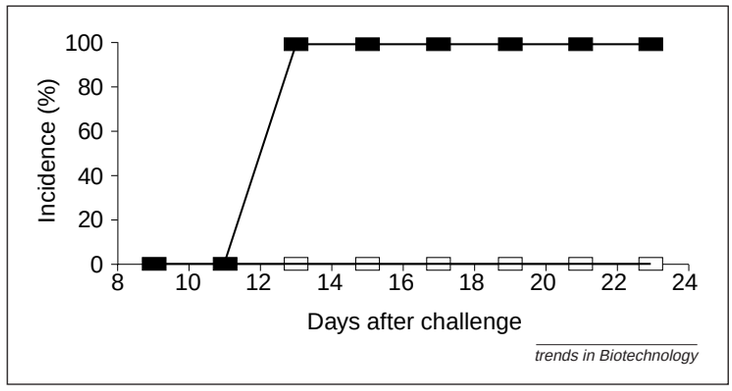

Figure 1

Cancer protection by Ii antisense-treated SaI/CIITA cells (open box) vs untreated SaI/CIITA cells (filled box). Sa murine sarcoma cells were stably transfected with the gene for human CIITA (creating SaI/CIITA cells). These cells expressed high levels of MHC class II molecules and Ii protein. The mice vaccinated with SaI/CIITA cells after anti-Ii antisense treatment were protected against challenge with parental tumor; mice vaccinated with comparable cells that did not have Ii protein suppression were not protected.

Ii protein is superinduced relative to MHC class II molecules in some tumors

An important and long-standing question in oncology is: how do tumor cells escape from immune surveillance? Several mechanisms have been proposed whereby tumor cells can remain non-immunogenic, even while expressing novel tumor antigens[19–26] (Table 1). Deletion of MHC class I alleles or the β-2 microglobulin gene, for example, make tumor cells incapable of presenting tumor antigens by MHC class I molecules. Similarly, deletion of transporters of antigenic peptides might block the pathway for the processing of tumor antigens into the ER[19,20]. Finally, deletion of the HLA-DM gene ensures that MHC class II molecules on tumor cells remain continuously occupied with cleaved leupeptin-induced peptide (CLIP); cells with this phenotype are incapable of presenting even exogenous tumor antigens[23,24].

Many normal tissues do not express MHC class II molecules or the Ii protein. The induction of MHC class II and Ii genes, however, occurs in many types of tumors including breast cancer[27–29], non-small lung cancer[30], colorectal adenocarcinoma[26,31–33], soft-tissue tumor[34], renal adenocarcinoma[35] and hairy-cell leukemia[36]. A common finding is that the Ii protein is expressed to the same or greater levels than MHC class II molecules. In a recent study, the level of Ii expression in human colon adenocarcinomas was shown to correlate positively with the severity of the anaplastic index and inversely with the index of tumor infiltrating lymphocytes[26]. This finding suggests that excessive Ii expression in some malignancies might block endogenous tumor antigen presentation by MHC class II molecules.

Ii protein prevents the presentation of endogenous antigens to CD4$^+$ T cells

Investigators have uncovered several functions of the Ii protein. One of the major functions of Ii is to protect the antigenic-peptide-binding site on MHC class II molecules from binding to endogenously derived antigenic peptides in the ER[37]. *In vitro* evidence has demonstrated that the binding of the Ii protein and

Table 1. Possible molecular mechanisms by which tumors might escape immune surveillance

Genetic changes	Possible mechanisms	Refs
TGF-β production and stroma formation	Inhibiting T-cell interactions with tumor cells	19
Selective deletion of MHC class I or β-2 microglobulin	Rendering tumor cells incapable of presenting tumor antigens by MHC class I molecules	20,21
TAP protein deletion	Blocking the transport of antigenic peptides (i.e. tumor antigens) into the ER	22
HLA-DM deletion	Inability to regulate antigenic peptide selection and exchange	23,24
Deletion of CIITA	Rendering tumor cells incapable of expressing MHC class II molecules	25
Superinduction of the invariant chain (Ii protein)	Increasing expression of the immunoregulatory Ii protein might alter endogenous tumor antigen presentation by MHC class II molecules	26, this review

Abbreviations: TGF, transforming growth factor; MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; ER, endoplasmic reticulum; CIITA, class II transactivating factor.

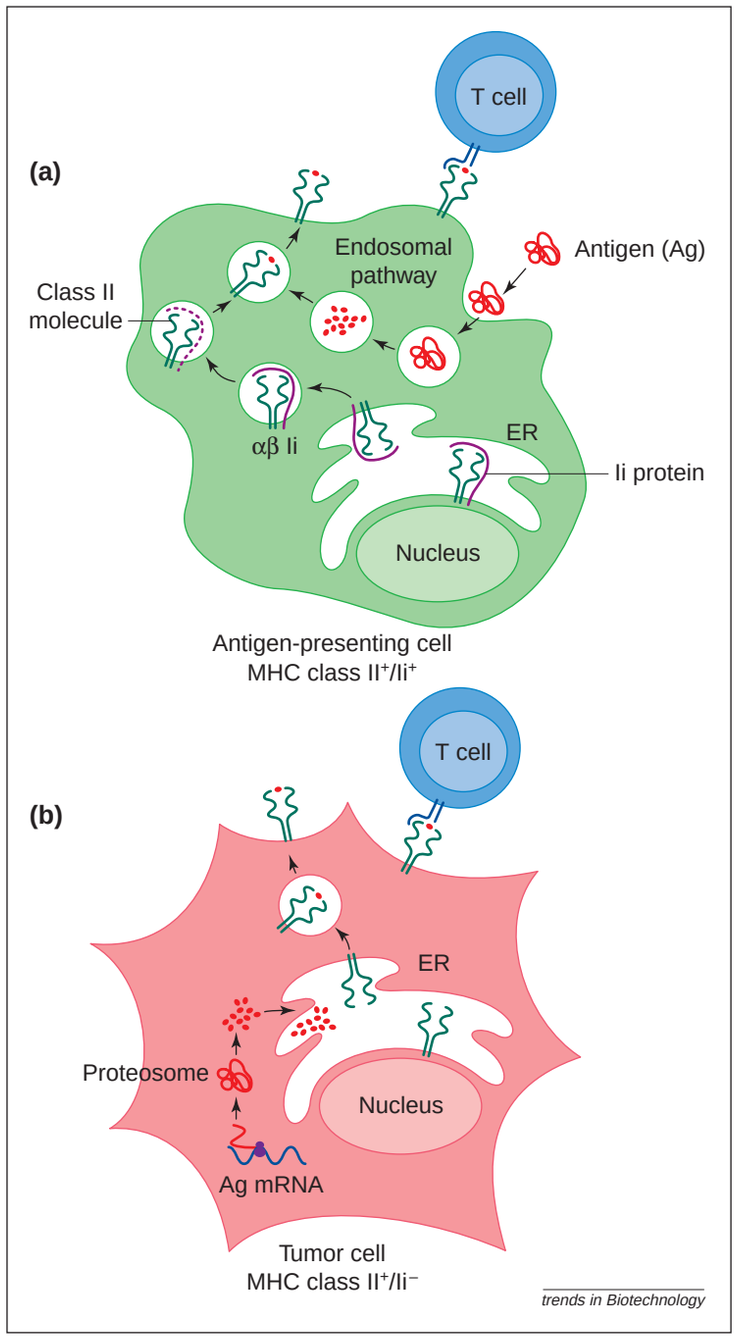

Figure 2

The control of antigen presentation by the Ii protein. The accepted current view of antigen processing and presentation of antigenic peptides by MHC class II molecules to T cells **(a)** is contrasted with alterations in tumor cells in which MHC class II molecules are present without the Ii protein **(b)**. In normal cells, the antigenic-peptide-binding site of MHC class II molecules is blocked by the Ii protein at synthesis in the endoplasmic reticulum (ER). Such complexes are transported to an antigenic-peptide-charging compartment wherein Ii protein is digested and released and antigenic peptide is charged, for transport to the cell surface. In the case of tumor cells with suppressed Ii protein, the MHC class II antigenic-peptide-binding site can receive ambient peptides, including tumor-related peptides (in the ER) from the repertoire of peptides surveyed by the MHC class I molecules that are charged with antigenic peptides.

antigenic peptides to MHC class II molecules is mutually exclusive. For example, Ii blocks the binding of synthetic antigenic peptides to purified DR molecules[38], and the binding of peptides to MHC class II molecules prevents those 'charged' MHC class II molecules from binding to Ii[39,40]. In living cells, the association of Ii with MHC class II molecules inhibits the binding of endogenous peptides to MHC class II molecules[41,42]. The diversity of MHC class II presented antigenic peptides is severely restricted by the Ii protein using a T cell proliferation assay[43]. Ii has been shown to prevent the HLA-DR-restricted presentation of an influenza matrix protein peptide synthesized in the cytoplasm[44] and to restrict the presentation of endogenous antigens in fibroblasts[45]. Using a panel of T cell clones, MHC class II-positive cells have been shown to activate different sets of clones depending on whether or not Ii is expressed[46]. In tumor cells, only Ii-negative SaI cells present ER-retained antigenic peptides[9,10,13]. Figure 2 depicts exogenous vs endogenous antigen presentation by MHC class II molecules in the presence and absence of Ii.

Another question that has been addressed is whether or not all MHC class II-positive but Ii-negative cells are capable of presenting endogenous antigens. In Ii-knock-out mice, the expression of MHC class II molecules is significantly decreased[47]. Ii protein is known to have a cytosolic intracellular signal for intracellular trafficking to the antigen charging compartment. The α chain of MHC class II molecules, however, also has an intracellular trafficking signal[48]. Several studies have suggested that the loading of endogenous peptides onto MHC class II molecules might be impaired in Ii-negative cells[49]. In the absence of Ii, large proteins including heat-shock proteins were found bound to MHC molecules. However, this does not exclude the binding of small peptides to MHC class II molecules[42]. It has been demonstrated in melanoma cells that the induction of MHC class II molecules by CIITA was insufficient to enable the presentation of antigens to T cells. This defect can be repaired by adding IFN-γ to the cell culture[50], suggesting that some proteins, in addition to those induced by CIITA, are involved in antigen processing and presentation. In summary, the Ii protein usually prevents MHC class II molecules from presenting endogenous antigens in most cases. However, in some cells, poor loading of peptides to MHC class II molecules occurs independently of the Ii protein, possibly as a result of the lack of unidentified proteins regulating antigen processing and presentation.

Inhibition of Ii results in a simple, effective and universal tumor-cell vaccine

A simple and effective method has been developed to inhibit Ii expression in MHC class II-positive tumor cells in which MHC class II molecules are either induced or constitutively expressed[18]. In the SaI murine sarcoma model, MHC class II-expressing SaI cells wherein the Ii protein was suppressed by antisense methods provided more-potent vaccination against a subsequent challenge with the parental tumor than did comparable cells in which Ii protein expression was not suppressed (Fig. 1). Because the Ii protein is mono morphic, these methods can be used in all patients with different MHC backgrounds. Further, Ii inhibition opens antigenic-peptide-binding sites on all MHC class II alleles, including HLA-DR, -DP, and -DQ. By contrast, transfecting patient's tumor cells with genes encoding MHC class II molecules is clinically impractical because of the high degree of polymorphism of

MHC class II genes. The use of only one MHC class II allele would limit the epitopes that can be displayed relative to the repertoire capable of being displayed by other MHC class II alleles. Given the great heterogeneity of MHC class II alleles in different cells, even within the same tumor, it is difficult to know which MHC class II allele can best present tumor antigens for immune stimulation. For these reasons, we believe that the induction of endogenous MHC class II molecules, such as using CIITA combined with Ii antisense, represents the simplest and most-effective way of generating a universal cancer-cell vaccine.

The selective expression of MHC class II molecules increases the repertoire of presented antigens, amplifies the immune response and increases immunological anti-tumor memory. These characteristics are mediated in large part by the activation of tumor-specific $CD4^+$ T cells[6]. An effective anti-tumor immune response requires activation of both $CD4^+$ and $CD8^+$ T cells. Activation of $CD8^+$ T cells is carried out primarily by cross priming[51], that is, through the presentation of tumor antigens by dendritic cells that have previously phagocytized dead tumor cells. By contrast, the activation of $CD4^+$ T cells occurs both by direct and cross priming of T cells (Fig. 3). Direct priming of tumor-specific $CD4^+$ T cells by MHC class II^+ and Ii^- tumor cells has been confirmed by us[18] and others using murine SaI sarcoma cells[9,10,12,13] and melanoma cells[52].

However, several questions remain that must be addressed before the full potential of the immunotherapy strategy described here can be assessed. Can all tumor cells present endogenous tumor antigens when they express MHC class II molecules in the absence of Ii? What other methods can optimally induce MHC class II molecules and synergize with Ii inhibition? Will the absence of other factors in antigen processing and presenting pathways continue to protect tumors in which we attempt to create vaccines by the methods reviewed here? We are now investigating the use of Ii antisense in other tumor models to understand the generality of this method.

To date, we have found that Ii antisense can effectively inhibit Ii expression without affecting MHC class II expression in examples of murine sarcoma, colon adenocarcinoma, melanoma and lymphoma, as well as human prostate adenocarcinoma and other human tumors. Inhibition of Ii can have an additive or even synergistic effect when combined with other methods of immune stimulation. For example, inhibition of Ii in SaI cells induced for MHC class II expression by transfection with the gene for interferon-γ, rather than CIITA, results in a stronger immunity than does Ii inhibition in CIITA-induced cells[18]. Thus, interferon-γ might act synergistically with Ii inhibition on the host immune system to generate more-effective protective immunity. There are a variety of other cytokines[5] and costimulatory molecules[4,52] that remain to be explored for use in conjunction with Ii inhibition to enhance T-cell priming by MHC class II^+ and Ii^- tumor cells.

In summary, creating tumor cells expressing MHC class II molecules without the immunoregulatory Ii protein leads to an effective cancer-cell vaccine. When tumor cells are MHC class II-positive, Ii protein, which is sometimes over-expressed, can be suppressed by antisense methods. When tumor cells are MHC class II-negative, expression of MHC class II and Ii protein can be induced with CIITA, or interferon-γ, and Ii protein subsequently suppressed by antisense methods. Additional preclinical tests are in progress to refine these methods for the immunotherapy of metastatic human cancers.

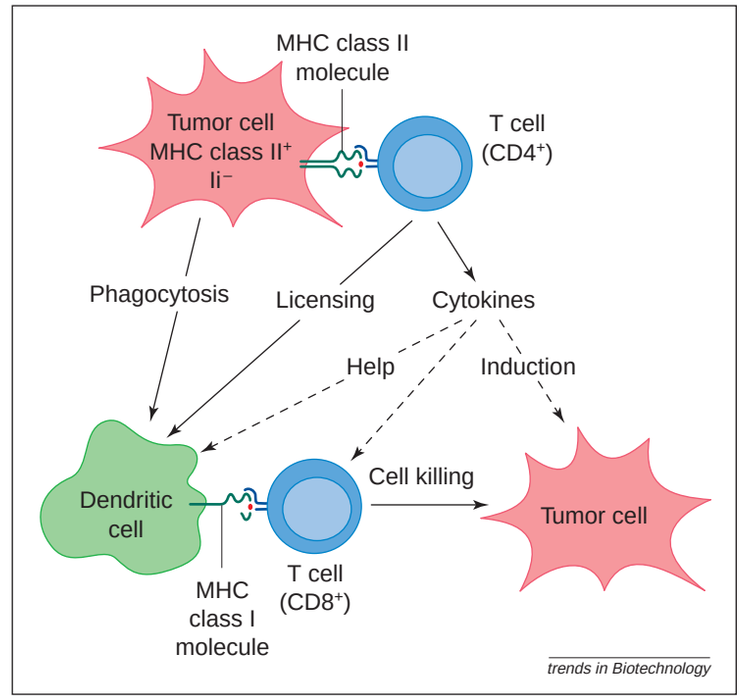

Figure 3

Priming of $CD4^+$ and $CD8^+$ T cells for anti-tumor activity. 'Licensing' of dendritic cells occurs by interaction with $CD4^+$ T cells that have themselves been activated by the presentation of antigenic determinants via MHC class II molecules on tumor cells. Those activated or licensed dendritic cells can then efficiently activate $CD8^+$ T cells, which recognize antigenic determinants presented by the MHC class I molecules of those dendritic cells. Tumor cells that are made MHC class II-positive, Ii-negative can activate $CD4^+$ T cells directly to enhance the potential of that population of cells to license dendritic cells. In addition, cytokines released by activated $CD4^+$ T cells facilitate cross priming of $CD8^+$ T cells and might induce expression of MHC class I and II molecules on tumor cells.

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