

review

MHC class II molecules and tumour immunotherapy

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Background. Tumour immunotherapy attempts to use the specificity and capability of the immune system to kill malignant cells with a minimum damage to normal tissue. Increasing knowledge of the identity of tumour antigens should help us design more effective therapeutic vaccines. Increasing evidence has demonstrated that MHC class II molecules and CD4⁺ T cells play important roles in generating and maintaining antitumour immune responses in animal models. These data suggest that it may be necessary to involve both CD4⁺ and CD8⁺ T cells for more effective antitumour therapy. Novel strategies have been developed for enhancing T cell responses against cancer by prolonging antigen presentation of dendritic cells to T cells, by the inclusion of MHC class II-restricted tumour antigens and by genetically modifying tumour cells to present antigen to T lymphocytes directly.

Conclusions. Vaccines against cancers aim to induce tumour-specific effector T cells that can reduce tumour mass and induce development of tumour-specific T cell memory, that can control tumour relapse.

Key words: neoplasms; immunotherapy, adoptive; CD4 – positive T – lymphocytes; T – lymphocytes, helper – inducer; cancer vaccines

Introduction

Immunotherapy denotes a strategy for manipulating a patient's immune response. In cancer or infectious disease the approach is designed to boost the patient's response to tumour antigens or pathogens.¹ Many strategies for enhancement of the immune response to autologous tumours have recently been deve-

loped. These strategies use tumour cells transfected with genes encoding molecules that enhance immune responses.² Tumour specific immunity is mediated by T lymphocytes. T cells play a major role in the antitumour immune response and surveillance and represent an important basis for the development of cancer immunotherapy.³ Identification of immunogenic tumour antigens has significantly advanced our understanding of tumour immunity and provides opportunity for the development of effective antigen-specific cancer therapy.⁴ Since most cancers do not express major histocompatibility complex (MHC) class II molecules on their surface and CD8⁺ cytotoxic T lymphocytes (CTLs) are able to induce lysis of tumour cells upon recogni-

Received 5 October 2005

Accepted 11 October 2005

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tion of tumour antigen derived peptides, presented by the tumour's MHC class I molecules, the research has been focused mainly on modulation and use of MHC class I antigen presenting pathway for tumour immunotherapy. However, clinical trials using MHC class I restricted antigens have elicited only modest and transient immune responses in most immunized patients. A possible reason for this failure is the lack of tumour specific CD4⁺ T cell responses⁵, so recently a lot of progress has been made in acknowledging the importance of MHC class II molecules in mediating antitumour immune response.⁶⁻⁸

MHC class I and MHC class II antigen presentation pathways

The MHC is a large multigene family that encodes cell surface glycoproteins involved in binding and presentation of antigenic peptides to T lymphocytes. MHC class I molecules, which are expressed on most nucleated cells, present peptides to CD8⁺ cytolytic T lymphocytes (CTLs). In contrast, the constitutive expression of MHC class II molecules, which are essential for antigen presentation to CD4⁺ T helper (T_H) cells, is restricted to antigen presenting cells, such as dendritic cells, B cells, monocytes, macrophages and thymic epithelial cells. Expression of MHC class II molecules can however be induced by interferon- γ (IFN- γ) on most other cell types.⁹ Class II molecules usually present exogenously synthesized peptides, which are acquired in the cellular compartment for peptide loading, whereas class I molecules usually present endogenously synthesized self-peptides.¹⁰ Endogenous antigens are degraded by proteasome into short peptides. These peptides are transported into the endoplasmic reticulum (ER) by TAP complex. Here the newly synthesized MHC class I heavy chains assemble with the light chain and peptide and this complex is transported to the cell surface for presentation.

on to CD8⁺ CTLs. MHC class II molecule is usually unable to bind endogenous peptides, because the peptide antigen binding groove is occupied by invariant chain (Ii) molecules in the ER. This assembly stabilizes the MHC II complexes and its CLIP region prevents the binding of endogenous antigen peptides present in the ER. Ii also contains two sorting signals in its cytoplasmic tail, which are responsible for the transport of the MHC/Ii complexes into endosomal and lysosomal compartments, where Ii is degraded by cathepsins and only CLIP peptide is left in the binding groove. HLA-DM then catalyses the release of CLIP, allowing the groove to bind the antigen-derived peptides, which come from the lysosome (Figure 1).^{5,11,12}

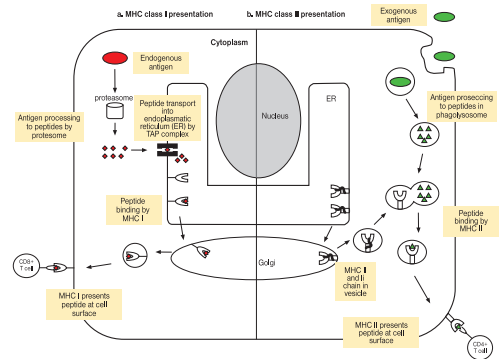


Figure 1. MHC class I and class II antigen processing and presentation pathways. (a) Proteasome degrades endogenous antigens into peptides, which are transported into the ER by TAP complex. Here the newly synthesized MHC class I molecules assemble with peptide and the MHC-peptide complex is transported through the Golgi to the cell surface for presentation to CD8⁺ T cells. (b) Exogenous antigens are taken in by endocytosis and processed by proteases in an endosome into short peptides. The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized, assembled in the endoplasmic reticulum, and transported through the Golgi apparatus to reach the endosome, where the invariant chain is digested, and the peptide fragments from the exogenous protein are able to associate with the MHC class II molecules, which are finally transported to the cell surface for presentation to CD4⁺ T cells.

The role of CD4⁺ T cells in immunity

CD4⁺ T lymphocytes play a central role in the onset and maintenance of adaptive immunity. CD4⁺ T cells help antibody responses and also help the activation and expansion of CD8⁺ T cells and are essential in maintaining the CD8⁺ T cell memory and long-lasting anti-tumour immune response (Figure 2).^{5,13}

CD4⁺ T cells can be divided into two main subsets: T_H1 and T_H2, depending on the cytokines they produce in response to antigen activation. T_H2 produce IL-4 and IL-5. IL-4 activates B cells to become antibody secreting plasma cells. IL-5 is a growth and activation factor for eosinophils. It has been reported that a significant cytotoxicity against tumour cells can be mediated by eosinophils after IL-5-mediated *in vivo* activation and that eosinophils may be involved in the antitumour response *in vivo*.¹⁴ T_H1 cells produce IL-2, IL-12 and IFN- γ , which are important for cellular immunity. IL-2 has been used in several studies in which its administration facilitates tumour eradication.^{13,15} IL-12 plays an essential role in the interaction between the innate and

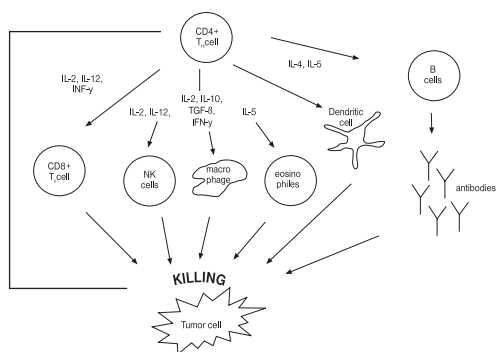


Figure 2. The role of CD4⁺ T cells in immune response against cancer. T_H cells play a crucial role in regulating the host immune response. They help B cells to produce antibodies, they release cytokines, which stimulate other varieties of immune cells to kill the invading tumour cell. They provide help in stimulating CD8⁺ CTL, which directly kill tumour cells. Finally, T_H cells are also involved in the inhibition of tumour growth in the absence of CD8⁺ T cells.

adaptive immunity. IL-12 acts on T cells and NK cells by inducing proliferation and production of cytokines, especially IFN- γ . IL-12 is also the major cytokine responsible for the differentiation of T_H1 cells, which are potent producers of IFN- γ . In experimental tumour models, recombinant IL-12 treatment has a dramatic anti-tumour effect on transplantable tumours, on chemically induced tumours, and in tumours arising spontaneously in genetically modified mice.¹⁶ IFN- γ also plays an important role in tumour rejection. IFN- γ could have direct effects on tumour cells by (a) cytotoxic activity on tumour cells, mediated by production of oxygen derivatives and nitric oxide, (b) up-regulation of MHC class II molecules expression, thus increasing tumour cell recognition and elimination, (c) alteration of the endogenous antigen-processing machinery, and (d) induction of inhibitors of angiogenesis in the cells.^{13,17}

The importance of CD4⁺ T cells in response to tumours and protection against tumour growth is now widely recognized. Strategies have evolved to generate tumour cells that can directly present tumour peptides and specifically activate tumour-specific CD4⁺ T_H cells. This approach is based on the assumption that the effectiveness of CD8⁺ T cells is dependent on sufficient help from tumour-activated CD4⁺ T cells, and that optimal immunological memory can be generated if both CD4⁺ and CD8⁺ T cells are stimulated.¹⁸

Tumour immunotherapy by modulating MHC class II gene expression in tumour cells

Down regulation of MHC class I or class II expression is one way for tumours to escape immunosurveillance. Whereas some tumours do express variable levels of MHC class II molecules, they often up-regulate expression of the Ii protein and thus prevent MHC class II presentation of endogenous tumour antigens.

Tumour cells that co-express class II and Ii molecules, such as B-lymphomas, are not capable of directly presenting tumour peptides and are thus no more immunogenic than class II negative tumour cells.¹⁹ Melanoma tumours also express MHC class II molecules, which can present tumour antigens. However, as these tumours lack co-stimulatory molecules that are necessary to activate naïve CD4⁺ T cells, such as B7 ligand, this may result in tumour antigen presentation and the induction of tumour antigen-specific CD4⁺ T lymphocyte anergy. Through these mechanisms the MHC class II molecules may participate in melanoma progression and immune escape.^{20,21} In contrast, high levels of MHC class II expression in gastrointestinal and breast cancers are often associated with better prognosis, showing the involvement of CD4⁺ T cells in protective immune response against the tumour.^{22,23} In fact several groups have published to successfully treat MHC class II negative tumours by converting them into MHC class II positive and thus making them APCs.^{7,24}

MHC class II gene expression is regulated mainly on the transcriptional level. One of the most important factors is the class II transactivator (CIITA), which acts as coactivator by virtue of its ability to interact with other components of the MHC class II enhanceosome, which are present on MHC class II promoters. CIITA is a non-DNA binding protein and controls constitutive and inducible MHC class II gene activation. Coinciding with MHC class II expression, the constitutive expression of CIITA is confined to APCs only, and CIITA expression can be induced by IFN- γ in various other cell types. The transcriptional regulation of human CIITA is controlled by at least three independent promoter units (CIITA-PI, -PIII and -PIV), each transcribing a unique first exon. These isoforms of the protein are cell type specific. CIITA-PI and CIITA-PIII are used for constitutive expression in dendritic cells and B cells, respectively. CIITA-

PIV has been the promoter shown to be predominantly IFN- γ inducible.²⁵

The first demonstration that lack of IFN- γ mediated induction of MHC class II antigens was caused by the absence of expression of CIITA was made in foetal trophoblast-derived tumour cell lines. Expression of CIITA following gene transfer resulted in the induction and subsequent cell surface expression of all isotypes of MHC class II molecules.^{9,26}

A variety of mouse tumours have been transfected with syngeneic MHC class II genes, and the resulting transfectants are very effective vaccines against subsequent challenge with the wild type class II-negative tumours.^{10,27}

Interestingly, the expression of other genes whose products are involved in the MHC class II antigen presentation pathway, such as invariant chain and HLA-DM molecules, although not absolutely depending upon CIITA, is strongly increased in the presence of CIITA. Some studies show that coexpression of Ii is required for expression of functional MHC class II molecules²⁸, while others show, that class II are functional in the absence of Ii^{29,30} and that coexpression of MHC class II and Ii correlates with poor tumour prognosis.³¹

Meazza *et al* show, that by modifying the murine mammary adenocarcinoma TS/A cell line by CIITA gene transfer, CIITA⁺ tumour cells express surface MHC class II molecules. Even though these cells also up-regulate the invariant chain mRNA and corresponding protein, CIITA⁺ tumour cells were rejected in syngeneic recipients and the capacity to be rejected correlated with the amount of CIITA-mediated MHC class II expression. Tumour rejecting mice also became resistant to the rechallenge with the wild type tumour. This rejection required both CD4⁺ and CD8⁺ cells.⁷ Other groups however show, that up-regulation of Ii chain expression converts an immunogenic tumour to non-immunogenic, that is highly malignant in autologous mice.^{32,33}

In vivo studies demonstrate that MHC class II⁺ /Ii⁻ tumour cells, and not host derived cells, were the predominant antigen-presenting cells for MHC class II-restricted nuclear antigens.³⁴ Due to allele heterogeneity, the transfection of genes for autologous MHC class II molecules is not practical clinically. Alternative approaches inducing expression of MHC class II molecules with transfection of CIITA or IFN- γ stimulation of CIITA expression and suppression of Ii protein by antisense methods using short oligonucleotides have been used successfully in several types of tumours. The cytotoxic effect can be enhanced by co-injecting the cells with IL-2 gene expressing plasmid, since IL-2 promotes T cell infiltration and activation against tumour antigens.³⁵ Intra-tumoural gene therapy can also be aided by radiation of tumours to enhance the therapeutic efficacy of intra-tumoural gene therapy for in situ induction of tumour-specific immune response.³⁶ There are several possible mechanisms for radiation enhancement of gene therapy, which include (a) slowing of the tumour growth, so that immunotherapy has time to develop, (b) radiation induced tissue damage mobilizes inflammatory cells in the tumour vicinity, (c) radiation limits suppressive immunoregulatory T cells and (d) radiation increases gene transduction efficiency and duration of expression of surviving tumour cells.

The advantage of the methods that include converting tumour cells into antigen presenting cells is not only killing of the cells directly contacted by tumour therapy, but also eliciting of an immune response which in turn eradicates tumour cells and deposits at both locoregional and distant sites.³⁶

Dendritic cells as tumour-antigen presenting cells

Dendritic cells (DCs) are the most potent antigen-presenting cells. They can present tu-

mour antigens to immunologic effector cells. MHC II molecules on DC surfaces play an important role in priming effector cells against tumour cells and their antigens, so they may be used to overcome tumour escape. DCs capture and process antigens in periphery, express lymphocyte co-stimulatory molecules, migrate to lymphoid organs, and secrete mediators to initiate immune responses.³⁷ DCs present peptides to naïve T cells and induce a cellular immune response that involves both CD4⁺ T_H1 cells and cytolytic CD8⁺ T cells. They can also stimulate humoral immunity by activating naïve and memory B cells.³⁸ Effective cancer vaccines will need to elicit both CD4⁺ IFN- γ producing and CD8⁺ cytotoxic T cell responses. Successful antitumour immunity will therefore depend on receipt by DC of maturation signals, which drive differentiation of naïve CD4⁺ and CD8⁺ T cells into T_H1/T_C1 effector cells.¹ Thus DCs represent a powerful tool for vaccination against tumour cells,^{38,39} but one has to consider, that immature DCs can induce tolerance and only mature DCs, which express co-stimulatory molecules on surface and produce inflammatory cytokines, induce effective antitumour immunity.¹

Marten *et al* demonstrate the transfection of CIITA gene into DCs, which strongly increases MHC class II expression. Transfection of the DCs with CIITA leads to an increase in antitumoural immunostimulatory capacity and therefore suggests the use of DCs in treatment of cancer cells.³⁹

DCs pulsed with tumour antigens have also been used in several studies. Such DCs have been successfully used in raising specific CD8⁺ T cells. Similarly this approach is also used to raise specific CD4⁺ T cells by loading them with MHC class II restricted antigens.^{38,40} Even though more and more tumour-restricted antigens are being identified, unfortunately most tumours still have no defined tumour antigens,³⁹ so this method has only limited applicability in clinical therapy.

Zhao *et al* show that short incubation of mRNA-transfected DCs with antisense oligonucleotides directed against the Ii chain enhances the presentation of mRNA-encoded class II epitopes and activation of CD4⁺ T-cell responses *in vitro* and *in vivo*. Immunization of mice with the antisense oligonucleotide-treated DCs stimulates a more potent and longer lasting CD8⁺ CTL response and enhances the antitumour efficacy of DC-based tumour vaccination protocols. Since vaccination with tumour mRNA-transfected DCs does not require the identification of the effective tumour antigens in each patient with cancer and is not limited by tumour tissue availability, this approach could represent a broadly useful method to augment antitumour T cell immunity alongside CD8⁺ T-cell immunity.³¹

Conclusions

Our current understanding of molecular mechanisms of cancer and tumour-specific immune responses has greatly benefited from the advances in molecular genetics and immunology. At the same time, the advances in recombinant DNA technologies have been made, that enable development of immunotherapy for the disease. Different immunotherapy strategies have proven to be very effective in animal models; however patients in most clinical trials conducted so far have elicited only weak and transient immune response. Therefore combination of different treatment strategies, such as gene therapy combined with cytokine treatment, radiation and/or chemotherapy will have to be considered.

The identification of MHC class I and class II-restricted tumour antigens has enabled development of methods for the targeting of either defined epitopes or whole antigens into the MHC pathway. Tumour cells can be genetically engineered to function as APCs, thereby facilitating the generation of tumour-specific immunity. The advantage of this me-

thod is that prior identification of tumour antigens is not necessary. By inducing a potent antitumour immune response, tumour cells throughout the body that are left behind after surgery or radiotherapy could be eradicated. By enabling induction of a potent CD4⁺ and CD8⁺ T cell antitumour immune response, the clinical outcomes in patients with cancer should be greatly improved.

DCs are also an attractive target for therapeutic manipulation of the immune system in cancer. By loading them with combined MHC class I and class II peptides, they can be used to immunize patients.

The combined use of MHC class I and class II-restricted tumour antigens, co-stimulatory molecules and cytokines that can be used to enhance immune responses represent an unprecedented opportunity for the development of new generation of effective cancer vaccines.

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