

Immune responses to tumour antigens: implications for antigen specific immunotherapy of cancer

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Abstract

Tumour associated antigens recognised by cellular or humoral effectors of the immune system are potential targets for antigen specific cancer immunotherapy. Different categories of cancer antigens have been identified that induce cytotoxic T lymphocyte (CTL) responses in vitro and in vivo, namely: (1) “cancer testis” (CT) antigens, expressed in different tumours and normal testis, (2) melanocyte differentiation antigens, (3) point mutations of normal genes, (4) self antigens that are overexpressed in malignant tissues, and (5) viral antigens. Clinical studies with peptides and proteins derived from these antigens have been initiated to study the efficacy of inducing specific CTL responses in vivo. Immunological and clinical parameters for the assessment of antigen specific immune responses have been defined—delayed type hypersensitivity (DTH), CTL, autoimmune, and tumour regression responses. Specific DTH and CTL responses and tumour regression have been observed after the intradermal administration of tumour associated peptides alone. Peptide specific immune reactions were enhanced after using granulocyte macrophage stimulating factor (GM-CSF) as a systemic adjuvant by increasing the frequency of dermal antigen presenting Langerhans cells. Complete tumour regression has been observed in the context of measurable peptide specific CTL. However, in single cases with disease progression after an initial tumour response, either a loss of single antigens targeted by CTL or of the presenting major histocompatibility complex (MHC) class I allele was detected, pointing towards immunisation induced immune escape. Cytokines to modulate antigen and MHC class I expression in vivo are being evaluated to prevent immunoselection. Recently, a new CT antigen, NY-ESO-1, has been identified on the basis of spontaneous antibody responses to tumour associated antigens. NY-ESO-1 appears to be one of the most immunogenic antigens known to date, with spontaneous immune responses observed in 50% of patients with NY-ESO-1 expressing cancers. Clinical studies have been initiated to evaluate the immunogenicity of different NY-ESO-1 constructs to induce

both humoral and cellular immune responses in vivo.

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Spontaneous immune responses against human tumours have been reported in different types of cancer, especially in melanoma and renal cell carcinoma,^{1,2} but also in other types of cancer, such as non-small cell lung cancer, bladder carcinoma, and breast cancer, indicating the specific interaction of the immune system with antigenic determinants presented by the tumour.^{3,4} The first evidence for antigen specific immune responses arose from studies with cultured melanoma cells, which were lysed by autologous CD8+ T cells in vitro in a major histocompatibility complex (MHC) class I restricted fashion. As a clinical extension of this observation, single patients with metastatic melanoma were vaccinated with irradiated autologous tumour cells for an extended period of time. Two patients (SK-29/MEL-AV and MZ-2/MEL-GH) with refractory metastatic melanoma have been followed by our group since 1978 and 1982, respectively.^{5,6} A complete regression of all tumour manifestations was achieved after prolonged immunisation with autologous tumour cells in both patients, which has been maintained for 20 and 15 years, respectively. In a collaborative effort, a systematic search was initiated to identify and characterise the cancer antigens and immune effector mechanisms mediating tumour regression in vivo.^{6–8}

Tumour antigens defined by specific T cell responses

CANCER TESTIS ANTIGENS

The specific lysis of cultured tumour cells by autologous cytotoxic T lymphocytes (CTL) in vitro was first observed in melanoma systems.⁶ Antigenic peptides presented by MHC class I and II molecules have been identified as the target structures for CTL recognition. The first antigen defined by CTL in the context of histocompatibility leucocyte antigen A1 (HLA-A1) was isolated from a melanoma cell line derived from patient MZ-2, designated MAGE-1.⁷ Later, a group of related genes (BAGE, GAGE) was identified, encoding antigens expressed in melanomas and several other tumours, but not in normal tissues except for the testis.^{7,9–12} Therefore, antigens of the MAGE pattern of expression are designated

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“cancer testis” (CT) antigens. More recently, a new CT antigen, NY-ESO-1, was cloned from an oesophageal cancer by the serological expression cloning method, an approach based on the screening of recombinant tumour cDNA libraries for specific interactions with autologous serum antibodies.^{13 14} HLA-A2 binding peptides derived from NY-ESO-1 were found that induce strong CTL responses *in vitro*.¹⁵ Because NY-ESO-1 and members of the MAGE gene family are frequently expressed in tumours of different histological type, they are attractive targets for antigen specific immunotherapy of cancer.

MELANOCYTE DIFFERENTIATION ANTIGENS

A second group of antigens, first cloned from the SK-MEL-29 system, expressed during melanocyte differentiation was identified as targets for autologous CTL in melanomas.¹⁶⁻¹⁹ Epitopes derived from self antigens such as Melan A/MART-1, tyrosinase, gp100/Pmel17, and gp75/TRP-1 have been found to be targets for CTL and tumour infiltrating lymphocytes (TIL) in the context of HLA-A2.1 and other MHC class I molecules.²⁰⁻²² Phase I clinical trials in patients with melanoma using antigenic peptides injected intradermally have shown that specific delayed type hypersensitivity (DTH) reactions can be elicited.²³ Granulocyte-macrophage colony stimulating factor (GM-CSF) used as a systemic adjuvant enhanced peptide related DTH reactions in single patients.²⁴ In contrast to phase I clinical trials with MAGE derived peptides, where peptide specific CTL were rarely identified, the induction of peptide specific CTL was often observed after immunisation with peptides derived from Melan A/MART-1 and tyrosinase.^{23 24} Furthermore, objective tumour regression was observed in single patients under continued immunisation.²³⁻²⁶

POINT MUTATIONS

Several cancer antigens are defined by point mutations of constitutive cellular proteins, leading to strong CTL responses against tumour cells in patients with cancer or experimental animals.²⁷⁻²⁹ In breast cancer, mutations of the p53 and Ras proteins have been reported. Humoral immune responses to the mutated and the wild-type proteins occurring spontaneously in patients with breast, lung, and gastrointestinal cancer have been detected.^{30 31} In women with a family history of breast cancer, antibody responses to p53 occur with a higher incidence than in controls (11% *v* 1%).³⁰ Because most anti-p53 antibodies detected are of the IgG type, a CD4+ T cell response to p53 can be predicted. In single patients with breast cancer with an overexpression of p53 in primary tumours, a proliferative CD4+ T cell response to wild-type p53 was demonstrated.³² These findings suggest that immune responses occur after the mutation of oncoproteins. These may also recognise non-mutated portions of the proteins. It is still unknown whether intracellular p53 is also presented at the cancer cell surface or in the extracellular cancer environment to serve as a target

for humoral and/or cellular effectors to mediate tumour regression.

Mutant p53 has been shown to induce specific CTL responses that mediate lysis of the transformed cells in animal models. In a murine sarcoma model, vaccination with p53 peptides combined with interleukin 12 (IL-12) has led to regression of p53 expressing advanced Meth A sarcomas.³³ In many human cancers, the accumulation of wild-type p53 in the cytosol is seen. It is assumed that p53 can be effectively presented by MHC class I molecules to elicit specific CTL responses. Therefore, immune responses against wild-type p53 may be of benefit in the treatment of cancers with p53 accumulation.

Ras mutations involve single amino acid substitutions, mostly at positions 12 and 61. These are less complex than in p53 and thus easier to evaluate. CD4+ and CD8+ T cell responses mediating tumour cell lysis can be induced by immunisation with mutant Ras peptides in animal models.³⁴ In humans, it remains to be determined whether wild-type or mutant Ras protein is a useful target for active or passive immunotherapy. In a small number of patients with metastatic pancreatic cancer, Ras specific proliferative T cell responses were documented after immunisation with MHC class I restricted Ras peptides.³⁵

Other mutation induced antigens defined primarily through CTL recognition—MUM-1 and mutated cyclin dependent kinase 4 (CDK4)—have been shown to be new peptide epitopes presented by MHC class I molecules. It remains to be determined whether these antigens will be useful targets for CTL based vaccines in a larger patient population.^{27 29}

OVEREXPRESSED SELF ANTIGENS

Many tumours abundantly express normal self proteins. The most extensively studied self antigens that are targets for active and passive immunotherapy are Melan A/MART-1, a melanocyte differentiation antigen present in melanoma and normal melanocytes, and HER-2/neu, a growth factor receptor overexpressed in 30% of breast and ovarian cancers and a variety of other adenocarcinomas.³⁰ Immune reactions directed against these antigens may result in the damage of normal tissues. However, preliminary experiences with peptide immunisation in patients with Melan A/MART-1 expressing melanomas have not shown adverse reactions directed to normal tissues, except for the development of vitiligo in single patients.²³ Spontaneous humoral and cellular immune responses in patients with HER-2/neu expressing tumours have been described. They may be amplified by appropriate immunisation strategies, possibly leading to tumour regression.³⁰

VIRAL ANTIGENS

Viral diseases are associated with different malignancies in humans—for example, Epstein-Barr virus (EBV) with Burkitts lymphoma,³⁶ hepatitis B and C viruses (HBV, HCV) with hepatocellular carcinoma,^{37 38} human papilloma virus (HPV) with cervical

and anal carcinoma,³⁹ and human T lymphotropic virus (HTLV) with T cell leukaemia. Independent of whether the viral infection is the oncogenic agent, it was shown that viral antigens are expressed in the associated tumours and can be used as targets for preventive or therapeutic vaccination.⁴⁰

Development of immunotherapeutic strategies

PEPTIDES DERIVED FROM CT ANTIGENS

Peptides derived from MAGE-1 and MAGE-3 have been used alone or combined with different adjuvants—GM-CSF and QS21—for immunisation in HLA-A1 positive patients with MAGE expressing tumours. Tumour regression has been observed in more than 30% of patients with melanoma after immunisation with the MAGE-3 derived, HLA-A1 restricted peptide.⁴¹ However, MAGE-3 specific CTL were not detected in response to the vaccine in these patients.⁴² In a subsequent study with systemic GM-CSF to improve antigen presentation by enhancement of CD1a+ dermal Langerhans cells, followed by intradermal administration of MAGE-1 and MAGE-3 peptides, a partial regression of liver and lung metastases was achieved in a patient with melanoma within three months of immunisation (E Jäger *et al*, unpublished data). Correlating with this remarkable clinical development, MAGE-1- and MAGE-3 specific CTL were detected in this patient, and these cells showed an increased frequency after immunisation. Currently, a subsequent phase I study is being initiated to evaluate immune reactions to peptide vaccination in patients with other MAGE expressing carcinomas, such as breast, bladder, non-small cell lung, and head and neck cancer.

MAGE-1 and MAGE-3 specific CTL were repeatedly detected in the peripheral blood of patient MZ2, whose melanoma cell line gave rise to the discovery of the MAGE gene family.^{7,10} This suggests that antigen specific CTL may be effective mediators of tumour regression because this patient experienced a complete regression of metastatic, MAGE-1, and MAGE-3 positive melanoma after repeated immunisation with irradiated autologous, MAGE-1/MAGE-3 expressing tumour cells. During the course of continued tumour cell vaccination, increased frequencies of CTL against autologous tumour cells were detected in the peripheral blood of this patient.⁴³ However, the specificity of CTL responses could not be determined at that time because the structure of the antigenic determinant(s) was unknown. The infrequent detection of CTL against MAGE genes in patients with MAGE expressing melanoma may be a consequence of either a low immunogenicity of MAGE genes, or a frequency of CTL precursors below the level of detection. Different methods for the assessment of MAGE specific CTL responses are being evaluated. A sensitive approach appears to be the ELISPOT assay, an enzyme linked immunosorbent assay that visualises direct antigen–T cell receptor interaction by staining of the spot-like release of interferon

γ (IFN- γ) or other cytokines by the T cell, interacting specifically with its target antigen.

TARGETING MELANOCYTE DIFFERENTIATION ANTIGENS

Tumour regression in single patients with melanoma has been achieved after adoptive transfer of TIL lines with specificity for gp100/Pmel17, tyrosinase, and gp75 derived epitopes, suggesting that melanocyte differentiation antigens are tumour rejection antigens.^{16,44,45} To study the effects of T cell interactions with melanocyte differentiation antigens in vitro and in vivo we undertook the following investigations. We compared the baseline CTL reactivity against HLA-A2 restricted peptides derived from melan A/MART-1, tyrosinase, and gp100/Pmel17 in HLA-A2 positive patients with melanoma and healthy individuals,⁴⁶ and determined CTL responses to melanoma associated peptides injected intradermally as a vaccine in HLA-A2 positive patients with melanoma.^{23,24} In addition, we compared changes of expression of melanoma associated antigens and peptide presenting MHC class I molecules in melanoma tissues showing regression or progression in the presence or absence of detectable antigen specific CTL responses in vivo.⁴⁷

First, the spontaneous CTL reactivity against melanoma associated peptides was determined in patients with melanoma and in healthy individuals. Baseline CTL reactivity against the differentiation antigens Melan A/MART-1, tyrosinase, and gp100/Pmel17 is frequently detected in patients with melanoma and in healthy individuals, without significant differences in intensity and frequency of CTL responses.^{46,48,49} In healthy individuals, Melan A/MART-1 specific CTL, which lysed Melan A/MART-1 positive melanoma cells were isolated from depigmented skin (vitiligo areas).⁵⁰ These findings indicate that CTL responses against self antigens may occur spontaneously, and might be amplified by appropriate vaccination.

Peptides derived from Melan A/MART-1, tyrosinase, or gp100/Pmel17 can induce DTH reactions and specific CD8+ CTL responses after intradermal immunisation. Objective clinical responses were found to be associated with measurable CTL responses to the vaccine. Major toxicity of the vaccine was not observed. However, some patients with favourable clinical picture developed vitiligo.^{23,24} In a single patient, a clonal expansion of a Melan A/MART-1 specific T cell receptor V β 16 was identified in T cell cultures stimulated with Melan A/MART-1 peptide, from Melan A/MART-1 specific DTH reactions, and from vitiligo areas after continued immunisation with Melan A/MART-1 peptide for five years.⁵¹

Dermal CD1a+ antigen presenting cells (APCs), such as Langerhans cells, can be enhanced and activated by GM-CSF in vivo.⁵² Combined administration of melanoma associated peptides and GM-CSF resulted in the amplification of DTH reactions and CD8+ CTL responses. Immunohistochemical characterisation of DTH reactions showed infiltrates of CD4+ and CD8+ T cells and a strong

expression of IL-2 and IFN- γ , suggesting the activation of CD4+ T helper type 1 (Th1) cells and CD8+ CTL by the immunisation peptides presented by MHC class I molecules of dermal APCs.²⁴

AUTOLOGOUS AND ALLOGENEIC WHOLE TUMOUR CELL VACCINES

Despite the increasing number of tumour antigens defined in different types of tumours, many investigators have returned to the approach of active immunisation using autologous or allogeneic tumour cells to mount immune responses in patients with cancer without knowing the antigenic repertoire of the individual disease. Tumour cell lysates, irradiated whole tumour cells, and fusion products of tumour cells and autologous or allogeneic dendritic cells have been used for the immunisation of patients with melanoma, breast, and renal cell cancer. Clinical responses of metastatic disease were reported in single cases, but detectable immune responses against the vaccines were difficult to document.^{53 54}

Immunoselection of antigen and MHC class I loss variants

Monoclonal antibodies used for immunohistochemical staining of melanocyte differentiation antigens expressed in melanoma tissues are an important prerequisite for studying the microheterogeneity of defined antigens in tumour lesions.^{55 56} In HLA-A2 positive patients with melanoma immunised with Melan A/MART-1, tyrosinase, and gp100 derived peptides combined with GM-CSF, we observed after an initial phase of tumour regression in some patients, progressive disease in the presence of detectable peptide specific CTL.⁴⁷ When compared with the initially described homogeneous antigen expression, biopsies taken from lesions in the phase of progressive tumour growth showed a highly heterogeneous distribution of antigens in response to increased peptide specific CTL reactivity. Furthermore, a loss of MHC class I molecules, as detected by immunohistochemistry, was found in single cases, and this is an additional mechanism of immune escape from antigen specific immunosurveillance.

Future clinical studies involving antigen specific T cell reactions in patients with cancer will consider the prognostic implication of the heterogeneity of MHC class I and tumour associated antigen expression in tumours for T cell based immunotherapy. Cytokines, such as IFN- γ or IL-12, will be evaluated in future clinical trials to show whether they can modulate the expression of antigens and antigen presenting molecules in tumour tissues.

Immunotherapy in cancer: perspectives

Different types of cancer expressing defined tumour associated antigens may become targets for immunotherapeutic interventions. The growing number of tumour antigens detected and the experience with peptide vaccination in malignant melanoma have set a solid basis for the development of more effective immunotherapeutic strategies in patients with cancer.

CT antigens are thought to be promising targets for specific CTL induced by peptide or protein vaccines. Spontaneous antibody responses to CT antigens detected in the sera of patients with cancer,⁵⁷ and the correlation of antibody titres with the course of the disease,⁵⁸ suggest the presence of antigen specific CD4+ T cells against peptides presented by MHC class II molecules on the surface of tumour cells.⁵⁹ The characterisation of these antigens as targets for CD4+ T cell responses will allow combined immunisation with MHC class I and II binding epitopes, potentially eliciting more effective immune responses.

Targeting viral antigens expressed by different types of cancer, such as cervical and hepatocellular carcinoma, by active immunisation is a strategy currently being evaluated in clinical trials. Although there is some evidence for specific immune responses to the vaccine, major clinical responses have not been achieved yet.⁶⁰ Because viral infection is thought to be a tumorigenic factor, immunisation against viral epitopes may have a preventive benefit in stages of premalignancy or even earlier after infection.⁶¹

Future perspectives of tumour vaccination are focused on the definition of more potent strategies of immunisation. Whole tumour proteins containing multiple, possibly relevant, antigenic epitopes may increase the chance of polyvalent B and T cell activation. Adjuvants might enhance the immunogenicity of peptides and proteins by activating costimulatory factors and mediating the production of cytokines.⁶² Dendritic cells loaded with peptides or proteins in vitro, or transduced with the relevant genes, might effectively stimulate both MHC class I and II restricted T cells in vivo.^{63 64} Cytokines have been found to play a key role in T cell activation. GM-CSF has been shown to induce long lasting Th1 and CD8+ T cell responses by the efficient induction of dendritic cells in vivo.⁶⁵ IL-12 is a potent activator of Th1 and CD8+ T cells. At low doses, it has been shown to mediate complete tumour regression when used as an adjuvant to immunisation with a mutant peptide of p53 in an animal model.³³ The identification of new tumour antigens will provide a broader basis for polyvalent immunisation to prevent the escape of antigen loss variants.⁶⁶ As the clinical effectiveness of cancer vaccination becomes more established, antigen specific immunotherapy might be considered as an alternative modality for adjuvant treatment of patients with cancer at high risk for recurrence.

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