Cancer Immunol Immunother (2006) 55: 63–67
DOI 10.1007/s00262-005-0673-6

SYMPOSIUM PAPER

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Dendritic cell-based cancer immunotherapy targeting MUC-1

Received: 9 December 2004 / Accepted: 4 January 2005 / Published online: 28 April 2005
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Abstract Vaccination therapy using dendritic cells (DC) as antigen presenting cells (APC) has shown significant promise in laboratory and animal studies as a potential treatment for malignant diseases. Pulsing of autologous DCs with tumor-associated antigens (TAA) is a method often used for antigen delivery and choice of suitable antigens plays an important role in designing an effective vaccine. We identified two HLA-A2 binding novel 9-mer peptides of the TAA MUC1, which is overexpressed on various hematological and epithelial malignancies. Cytotoxic T cells generated after pulsing DC with these peptides were able to induce lysis of tumor cells expressing MUC1 in an antigen-specific and HLA-restricted fashion. Within two clinical studies, we demonstrated that vaccination of patients with advanced cancer using DCs pulsed with MUC1 derived peptides is well tolerated without serious side effects and can induce immunological responses. Of 20 patients with metastatic renal cell carcinoma, 6 patients showed regression of metastases with 3 objective responses (1 CR, 2 PR). Furthermore, we found that in patients responding to treatment T cell responses for antigens not used for treatment occurred suggesting that antigen spreading in vivo might be a possible mechanism of mediating antitumor effects. These results demonstrate that immunotherapy in patients with advanced malignancies using autologous DCs pulsed with MUC1 derived peptides can induce immunological and clinical responses. However, further clinical studies are needed to identify the most potent treatment regimen that can consistently mediate an antitumor immune response in vivo.

This article is a symposium paper from the conference "Progress in Vaccination against Cancer 2004 (PIVAC 4)", held in Freudenstadt-Lauterbad, Black Forest, Germany, on 22–25 September 2004.

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Introduction

The development of effective cancer vaccines relies not only on the identification of tumor-associated antigens (TAA), but also on the choice of antigen source as well as the optimal route of antigen delivery and using of adjuvants [1]. One of the most promising approaches to cancer immunotherapy is the administration of antigen-presenting cells (APC) such as dendritic cells (DC) loaded with TAA. DCs are the most potent APCs expressing high levels of major histocompatibility complex (MHC) and various immunomodulatory proteins and capable of sensitizing T cells to new and recall antigens [2]. Several techniques were established to load DCs with TAA. Peptides derived from endocytosed tumor lysates can be presented on MHC molecules after proteolytic processing [3]. RNA encoding for TAA or derived from tumor cells can be used to generate TAA in DC themselves [4]. One of the most common ways of generating tumor-specific immune responses is pulsing of DC with human leukocyte antigen (HLA) class I- and class II binding peptides, which are able to bind directly to the MHC molecule on the cell surface [5, 6].

MUC1 mucins are highly glycosylated type I glycoproteins expressed by various normal and malignant epithelial cells such as breast, kidney and ovarian cancers and hematologic malignancies including acute myelogenous leukemia (AML), multiple myeloma and some B-cell lymphoma [7–9]. The complex molecule is anchored within the cell surface by a transmembrane domain. The largest part is a unique extracellular domain with a variable number of tandem repeats (VNTR) of 20 amino acids [10, 11]. The overexpression of MUC1 in many malignancies makes it an attractive and broadly applicable target for cancer vaccination therapies [12,

13]. Previous studies have shown that the tandem repeat of MUC 1 is highly immunogenic and that peptides derived from this domain are recognized by cytotoxic T cells [14, 15]. Furthermore, it has been demonstrated that epitopes derived from this domain can initiate antibody-mediated immune responses [16, 17].

The majority of known TAA epitopes are presented within MHC class I molecules and are recognized by cytotoxic T cells (CTL), whereas a small number of TAA peptides are presented in association with MHC class II molecules and recognized by CD4+ T cells [18–20]. Most of TAA peptides used in cancer vaccination trials bind HLA-A2, the most common MHC class I molecule among Caucasians. The characterization of MHC class I allele-specific motifs to define epitopes contained within a given antigen was an important step in the development of effective cancer vaccines [21, 22].

Identification of MUC1 derived epitopes

For identification of MUC1 derived epitopes, which can be used for dendritic cell-based vaccination therapies, we screened the MUC1 protein for HLA-A2-binding peptides using a computer-assisted analysis [23, 24]. Two novel 9-mer peptides, M1.1 and M1.2, could be identified. Both peptides showed a high binding probability to HLA-A2. The M1.1 peptide is derived from the tandem repeat region of the MUC1 protein whereas M1.2 is localized within the signal sequence of MUC1 [25, 26]. To analyze whether these epitopes are presented by tumor cells endogenously expressing MUC1, we induced MUC1 peptide-specific CTL responses by primary in vitro immunization and used these CTL to determine the presentation of MUC1 epitopes on human tumor lines. DC were pulsed with M1.1 and M1.2 and demonstrated a high ability to initiate an MUC1-specific CTL responses. Incubation with the Pan-DR binding peptide PADRE was able to amplify the antigen-specific cytotoxic activity of the induced CTL [27].

In previous studies, it was shown that pulsing human lymphocytes with a liposome-encapsulated MUC1 peptides consisting of 25 amino acids from the VNTR could induce cytotoxic T-cell responses [28]. Comparable to the results obtained in our experiments MUC1 peptide-specific cytotoxic T-cell responses as well as the secretion of IFN-gamma were detected when the in vitro induced CTL were analyzed for their effector functions. In our study, we demonstrated that CTL were able to recognize tumor cells endogenously expressing the MUC1 protein in an HLA-A2-restricted manner and lysed cell lines of breast, pancreatic and renal cell cancer expressing MUC1 and HLA-A2. The cytotoxicity against tumor cells could be inhibited by cold HLA-A2+ targets pulsed with the cognate peptide in a cold-target inhibition assay and by anti-HLA-A2 MoAb.

An increased expression and secretion of the MUC1 protein is an independent predictor of poor prognosis and early metastatic disease in cancer patients [29, 30]. It was shown that the MUC1 protein can induce apoptosis of activated T cells in vitro [31]. Furthermore, it has been demonstrated that cancer associated MUC1 inhibits T-cell proliferation [32]. This inhibition was mediated by the whole MUC1 protein or large synthetic tandem repeats of the MUC1 core peptide and was reversible by addition of IL-2, anti-CD28 MoAb or short 16-amino acid MUC1 peptide, indicating that vaccination therapy using short synthetic peptides in combination with DC and/or IL-2 may overcome the observed immunosuppression in cancer patients. These findings were supported by studies with MUC1 transgenic mice, where the tolerance to human MUC1 antigen could be reversed by immunizing the animals with fusions of DC- and MUC1-expressing tumor cells [33].

Our results showed that the use of MUC1-derived peptides for a DC-based active specific immunotherapy could reverse the observed immunosuppression and MUC1 tolerance in cancer patients and provide an additional, broadly applicable approach to established therapies of epithelial malignancies, such as renal cell, breast, and pancreatic carcinoma.

Vaccinations with peptide pulsed dendritic cells. Clinical and immunological responses

In a phase I/II study, we analyzed the feasibility and efficacy of HER-2/neu or MUC1 peptide-pulsed ''mature'' monocyte derived DC vaccinations in heavily pretreated patients with metastatic cancer [34]. All patients had histologically confirmed metastatic breast or ovarian cancer that expressed HER-2/neu or MUC1. Ten patients with advanced diseases that were pretreated by multiple cycles of chemotherapy, including high-dose chemotherapy and autologous stem cell transplantation were included in this study. DC were pulsed with two HLA-A2 binding peptides deduced from MUC1 and two derived from the HER-2/neu protein (E75 and GP2) [35, 36]. Vaccinations were performed sc every 2 weeks four times and repeated afterwards monthly until tumor progression. The vaccinations were tolerated with no side effects. After three vaccinations, peptide-specific CTL could be detected in 5 of 10 patients in the peripheral blood using both intracellular IFN-gamma staining and ^{51}Cr-release assays, suggesting that even after high-dose chemotherapy TAA-pulsed DCs can induce antigen-specific immune responses. The main immunologic responses in vivo were induced with the HER-2/neu-derived E75 and the MUC1-derived M1.2 peptide, which lasted for more than 6 months, suggesting that these two peptides might be immunodominant. In one patient treated with MUC-1 peptide-pulsed DC, CEA- and MAGE-3 peptide-specific T-cell responses were detected after several vaccinations and MUC1 peptide-specific T cells were observed in another patient after seven

immunizations with HER-2/neu-derived peptides, suggesting that antigen spreading in vivo might occur after successful immunization with a single T cell epitope.

One possible mechanism for this kind of antigen spreading might be the induction of other tumor antigen-specific CTL as a result of the destruction of the malignant cells by the in vivo-induced CTL and uptake and processing of the killed cells by APC, such as DC or macrophages [37–39]. In this report, we showed for the first time that vaccination therapy using DC pulsed with HER-2/neu- or MUC1-derived peptides can induce immunologic responses in patients with advanced metastatic breast and ovarian cancers.

In the subsequent study, we analyzed the clinical and immunological responses in patients with metastatic renal cell carcinoma (RCC) using autologous mature monocyte derived DC pulsed with the HLA-A2 binding MUC1 peptides.

Therapy of metastatic RCC is still challenging because of its resistance to conventional therapies such as radiation or chemotherapy [40]. In view of the observed spontaneous remissions of advanced RCC and infiltration of cancer tissue with lymphocytes and dendritic cells, immune mechanisms have been suggested to play a role in the natural disease course of RCC and immunotherapy strategies like interleukin-2 (IL-2) and interferon-α (IFN-α) were developed [41, 42]. However, therapy is often only moderately tolerated and response rates of 20–30% remain unsatisfactory [43].

This has lead to a proliferation of clinical trials testing the effectiveness of DC-based immunotherapy in patients with advanced RCC [44–47]. We vaccinated 20 patients with metastatic RCC with DC loaded with the MUC1 derived peptides M1.1. and M1.2. For the activation of CD4+ T-helper lymphocytes, DC were further incubated with the PAN-HLA-DR binding peptide PADRE.

Vaccinations were performed sc every 2 weeks four times and repeated afterwards monthly until tumor progression. After the fifth DC injection, patients additionally received three injections/week of low dose IL-2 (1Mio IE/m^2) sc. In six patients, regression of metastases was induced with 3 objective responses (1 CR, 2 PR) and two mixed responses. Four patients had a stabilization of the disease. The enhancement of T cell precursor was monitored using IFN-γ ELISPOT and ^{51}Cr-release assays. MUC1 peptide specific T cell responses in vivo were detected in the PBMC of all patients with objective responses. These in vivo induced CTL were able to recognize target cells pulsed with the cognate peptide or matched allogeneic tumor cells of the cell line A498 (RCC, MUC1+, HLA-A2+) constitutively expressing MUC1 in an antigen and HLA restricted manner after in vitro restimulation. Similar to the preceding study, we could demonstrate that patients responding to the treatment developed T cell responses to HLA-A2 restricted epitopes not used for vaccinations like adipophilin, telomerase or OFA indicating that epitope spreading might occur. Proliferative responses to the PADRE peptide were detectable in 11 out of 16 analyzed patients, in some patients already after the first 2–3 vaccinations.

Conclusion

DC-based vaccination therapy for patients with malignant diseases has been a focus of intense research over the last years. Several different techniques of antigen loading of DC were established, the most widely used being incubation of DC with HLA-binding tumor-associated peptides. We were able to identify two HLA-A2 restricted peptides from the MUC1 protein and generated MUC-1 specific CTL, which lysed tumor cell endogenously expressing MUC1 in an HLA-A2 restricted fashion in vitro. We demonstrated that vaccination therapy in patient with advanced RCC can induce immunological and clinical responses. Irrespective of the small number of patients treated in these trials, the findings are encouraging and warrant further studies of tumor vaccination, particularly in patients with limited disease.

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