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ImMucin: A novel therapeutic vaccine with promiscuous MHC binding for the treatment of MUC1-expressing tumors

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ABSTRACT

An optimal cancer vaccine should be able to induce highly potent, long-lasting, tumor-specific responses in the majority of the cancer patient population. One approach for achieving this is to use synthetic peptide vaccines derived from widely expressed tumor-associated antigens, that promiscuously bind multiple MHC class I and class II alleles. MUC1-SP-L (ImMucin, VXL100) is a 21mer peptide encoding the complete signal peptide domain of MUC1, a tumor-associated antigen expressed by over 90% of solid and non-solid tumors. MUC1-SP-L was predicted in silico to bind various MHC class I and MHC class II alleles, covering the majority of the Caucasian population. PBLs obtained from 13 naïve donors all proliferated, with a Stimulation Index (SI $\geq$ 2), to the MUC1-SP-L peptide, producing mixed $CD4^+$ and $CD8^+$ responses. Similar results were manifested by MUC1-SP-L in PBLs derived from 9 of 10 cancer patients with MUC1 positive tumors. $CD4^+$ and $CD8^+$ T cell populations exhibited CD45RO memory markers and secreted IFN-gamma and IL-2 following stimulation with MUC1-SP-L. These T cells also exhibited proliferation to the MUC1-SP-L inner 9mer epitopes and cytotoxicity against tumor cell lines expressing MUC1 and a concordant MHC class I allele. Cytotoxicity to MUC1-expressing human and murine tumors was shown also in T cells obtained from HLA-A2 transgenic mice and BALB/c syngeneic mice immunized with the MUC1-SP-L and GM-CSF. In an immunotherapy model, BALB/c mice inoculated with metastatic MUC1 transfected murine DA3 mammary tumor cells, exhibited significantly prolonged survival following vaccination with MUC1-SP-L. Our results indicate superior immunological and anti-tumor properties of MUC1-SP-L compared to previously published MUC1-derived epitopes.

1. Introduction

Anti-tumor vaccination using tumor-associated antigens (TAAs) is considered a safe and specific methodology for controlling metastases in cancer patients [1–3]. The rationale behind this approach is that cancer cells that express TAAs in association with their MHC molecules can be subject to recognition and destruction by tumor-specific T cells. However, since these vaccines are often derived from less-immunogenic self-TAAs and are used to treat sick individuals, frequently with compromised immune systems, they should be able to induce a strong and preferably a broad immune response [4].

One approach used to improve a specific immune response was to construct a multi-epitope vaccine that makes use of a mixture of specific MHC class I epitopes derived from different TAAs [5,6]. However, even the enhanced cytotoxic T lymphocytes (CTL) antigenic repertoire in such vaccines could not compensate for the lack of pan-HLA response [7], since they usually comprised of a single MHC class I-restricted epitope. Moreover, these vaccines also lacked MHC class II restricted T helper epitopes. In a few cases, the lack of MHC class II epitopes in such vaccines was shown to induce immunological tolerance to the immunizing antigens [8], rather than long lasting immunity mediated via $CD8^+$ T cell activation. In the past, the limited number of known TAA-derived MHC class II epitopes has led to the use of non-specific 'universal' MHC class II-restricted epitopes [9]. In one report, the use of a pan-HLA class II epitope peptide (PADRE) increased the response against the helper

Abbreviations: CTL, cytotoxic T lymphocytes; DC, dendritic cells; mAb, monoclonal antibody; SI, stimulation index; SP, signal peptide; TAA, tumor-associated antigen; TRA, tandem repeat array; VCs, vaccine candidates.

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epitope, but the elevation in CD8+ T cell effectors specific to the MHC class I-restricted epitope was limited [10].

Another approach to improving a specific immune response was to use longer peptides with promiscuous MHC binding properties. There is increasing evidence that vaccines combining MHC class I and class II epitopes, potentiate better anti-tumor effector function and long-term immunity [4,11]. In that setting, antigen specific CD4+ T cells can activate dendritic cells (DC), principally through the interaction of CD40–CD40L. The "conditioned" DC can in turn activate tumor specific CD8+ T cells and cross-present tumor epitopes to them at the tumor site or at remote locations [11–15]. Recent studies, primarily on the Her-2/Neu [16], RAS [17], and NY-ESO-1 [18] TAAs and the human papilloma virus 16 derived E6, E7 [19] antigens, showed that strong, long-term cellular immunity can be induced via longer peptides that encode on a single sequence a combination of one, or less frequently two, CD4+ and/or CD8+ epitopes derived from the same antigen. We recently showed that entire SP domains, usually comprised of 15–40mer long peptides, are inherently immunogenic, owing to their high hydrophobicity, and to their unique sequence motifs (20). This profile makes them a preferred T cell vaccine candidates (VCs) with antigen specific CD4+ and CD8+ epitopes [20]. SPs are usually found in the N-terminus of proteins and share an organelle-related common motif. Nevertheless, different SPs of various antigens exhibit high sequence variability with no particular sequence identity while conforming to the motif needed to maintain their functionality; thereby, they can serve as an ideal VC [20–23].

Following this rationale, our goal in this study was to select and develop a peptide vaccine from the MUC1 TAA that harbors on a single sequence multiple epitopes with more than 50% HLA binding coverage for the Caucasian population, for both CD4+ and CD8+ T cells.

MUC1 is a high-molecular-weight glycoprotein that is overexpressed in a broad range of solid and non-solid tumors [24,25]. The association of MUC1 with cancer progression has been well documented for the past three decades [26–28]. However, the importance of gene amplification as a mechanism leading to increased MUC1 expression in cancer has not been well characterized. Recent studies described the role of MUC1 gene amplification and protein expression in cancer development [29]. Results indicate that MUC1 copy number increases from normal tissue to primary invasive breast carcinomas in correlation with MUC1 protein expression [29]. These results suggest that MUC1 is a preferred TAA for anti-tumor vaccination. Initially, most anti-MUC1 vaccines were directed against the highly immunogenic, extracellular tandem repeat array (TRA), which is under-glycosylated during malignancy, and thereby was considered to expose new TAA epitopes [30]. Although immunity to TRA epitopes was shown to induce antibodies and MHC restricted CTLs [30], studies reported inconclusive results regarding its efficacy in anti-tumor vaccinations [30–33]. Likewise, there is no consensus concerning the correlation between the changes in exposure of sugar moieties and the improved recognition and function of CTLs [34]. More recently, MHC class I epitopes surrounding the TRA and within the SP of MUC1 have also been identified [35]. Experiments with the SP-derived 9mer HLA-A2.1 restricted epitopes MUC1D6 [36] and M1.2 [37] supported the notion that MUC1-SP-derived epitopes associated with MHC molecules are highly expressed on the surface of cancer cells. These epitopes also manifested anti-MUC1 and anti-tumor immunity in mice, as well as safety results in cancer patients [34,36–38].

In the present study, we isolated MUC1-SP-L (ImMucin, VXL100), a 21mer peptide vaccine encoding the entire SP domain of the MUC1 TAA. MUC1-SP-L was selected due to its in silico based promiscuous binding properties to both MHC class I and class II alleles. MUC1-SP-L encodes a number of known MHC class I, 9mer epitopes such as the MUC1D6 [36] (MUC1-SP-S1), M1.2 [37] (MUC1-SP-S2) and several novel epitopes such as MUC1-SP-S4 and MUC1-SP-S5. Our results with MUC1-SP-L showed broad HLA binding and robust antigen specific activation of human CD4+ and CD8+ T cells obtained from healthy volunteers and cancer patients bearing various MUC1 positive tumors. Moreover, MUC1-SP-L induced MUC1-specific CTL response in both syngeneic BALB/c mice and transgenic HLA-A2.1 (HHD-2) mice. The cellular response to MUC1-SP-L in mice was superior to that of the known TRA epitope BLP-25 (MUC1-TRA-L) [39,40]. The use of vaccines such as MUC1-SP-L that induce a strong and a broad T cell response could potentially be translated to better outcomes when using immunotherapy to treat cancer.

2. Materials and methods

2.1. Mice

The derivation of HLA-A2.1/D^b-β2 single-chain, transgenic, *H-2D$^{b-/-}$xβ2M$^{-/-}$* double knockout mice (named HHD-2 mice) has been previously described [14]. Eight- to 12-week-old HHD-2 mice were bred at the Weizmann Institute of Science breeding facility.

Six- to 8-week-old BALB/c mice were bred at the Tel Aviv University breeding facility. All experiments were conducted according to Weizmann Institute of Science and Tel Aviv University institutional rules and regulations.

2.2. MHC binding predictions

Binding predictions were performed for HLA class I (HLA-A, B, C) and HLA class II (HLA-DRB1) alleles that are most frequent worldwide. However, in order to have a defined population, in this study we focused only on the Caucasian population. The binding strength of 9mers to the class I alleles was predicted using BIMAS, http://www.bimas.cit.nih.gov/molbio/hla_bind/ [41]. The prediction of HLA class II peptide binding was done using Propred, http://www.imtech.res.in/raghava/propred/ [42] and Immune Epitope, www.immuneepitope.org [43]. We defined different binding strengths for class I as Strong = peptide score of >100, Medium = 10–100, Weak = 5–10.

Binding strength for DR HLA class II binding was defined in Propred as Strong = top 1% of binders, Medium = 1–2% of binders, Weak = 2–3% of binders. In Immune Epitope, for HLA-DRB1-0901, Strong = IC50 of 0.01–9.9 nM, Medium = 10–99.9 nM, and Weak = 100–10,000 nM.

2.3. Tumor cells

MDA-MB-231 human breast cancer and U266 human multiple myeloma are cell lines positive for both HLA class A2.1 and MUC1. MDA-MB-468 is a human breast cancer line, which is negative for HLA-A2.1, but positive for MUC1 expression. The K-562 human myalo-leukemia cell line and the human MOLT-4 T cell leukemia cell line express neither HLA-A2.1 nor MUC1. The 721.221 B-lymphoblastoid cell line is a TAP-2-deficient lymphoma clone of human origin which is negative for MUC1 expression. The 721.221-A2.1 cell line is the wild type 721.221 line stably transfected with HLA-A2.1 [44]. All cell lines were maintained in RPMI-1640 (Biological Industries, Beit Haemek, Israel) medium supplemented with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, 1% non-essential amino acids and 50 μg/ml gentamycin (Biological Industries, Beit Haemek, Israel) (termed here, complete medium). DA-3 is a metastatic cell line selected from the DMBA-induced mammary tumor in BALB/c mice [45]. DA-3TM are hMUC1 transected DA-3 cells [45].