

Mimotope vaccines for cancer immunotherapy

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Abstract

Cancer vaccines need to be designed to effectively induce tumor-specific CD8⁺ T cells, the key effector cells in immune responses against tumors. These T cells recognize peptides generated from cellular proteins by limited proteolysis, and bound and presented at cell surfaces by MHC class I molecules. Mimotopes, mimetics of T cell epitopes, have been derived from known epitopes by sequence modification, or developed *de novo* using combinatorial peptide libraries to scan the entire sequence space for peptides that induce the desired T cell responses. Mimotopes of both types have been tested in clinical vaccination trials for treatment of cancer.

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1. Introduction

The identification of tumor-associated antigens (TAA) is of major importance for the development of tumor antigen-specific therapeutic and prophylactic vaccines, and prerequisite for monitoring of tumor-specific immune responses, including the immunological effects of therapeutic interventions. The knowledge of tumor-associated antigens provides insights into the physiological state of the tumor cells and information on the interrelationship of tumor and immune system and, thereby, adds to our understanding of cancer. Cytotoxic T lymphocytes (CTL) play a central role in the immune surveillance of the disease. They can utilize different mechanisms to destroy tumor cells including perforin

and granzyme-mediated cytotoxicity. The cytotoxic responses of the CTL are triggered by recognition of peptides termed T cell epitopes that are derived from TAA by limited proteolysis, and bound to and presented by major histocompatibility complex (MHC) molecules (in man, human leukocyte antigens, HLA) at the surfaces of the cells. In recent years, a number of tumor-associated T cell epitopes (TATE) have been identified by employing different technologies including: (1) direct isolation of MHC-bound peptides that stimulate TAA-specific T cells, separation of these peptides by high resolution liquid chromatography (HPLC) and determination of their sequences by mass spectrometry; (2) T cell-guided epitope mapping of known or suspected TAA with overlapping peptides or peptides predicted to bind to the MHC molecules; (3) identification of the genes coding for tumor-associated antigens by screening with T cells cDNA expression libraries transfected into appropriate target cells, and subsequent epitope mapping; (4) identification of potential antigens using serological techniques such as seroscreening of phage expression libraries of cDNA of tumor cells (SEREX) followed again by epitope mapping [1].

To date some 300 different TATE representing some 100 different TAA have been reported [1]. The TAA can be classified by their expression patterns and source as, first, differentiation antigens expressed by tumor cells and

Abbreviations: APC, antigen presenting cell; BFA, brefeldin-A; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr-Virus; FCS, foetal calf serum; HLA, human leukocyte antigen, the human MHC; IFN, interferon; MHC, major histocompatibility complex, in humans: HLA; PBMC, peripheral blood mononuclear lymphocyte; SEREX, serological analysis of tumor antigens by recombinant cDNA expression cloning; TAA, tumor-associated antigen; TATE, tumor-associated T cell epitope; TCR, T cell receptor

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normal cells of the tissue type of the tumor; second, cancer testis antigens expressed by tumor cells and testis, an immune-privileged organ what makes these antigens relatively tumor-specific; third, embryonic antigens expressed by tumor cells and cells of earlier differentiation stages of the tissue from which the tumor arose; fourth, tumor-specific mutations including idiootype sequences in cases of lymphomas; fifth, viral antigens in cases of cancers with viral etiology [1]. Of all these categories, only mutated proteins are truly tumor-specific neoantigens but they are individualized antigens restricted to a specific tumor of an individual patient. Antigens of oncogenic viruses are foreign antigens and can be targeted just as any antigen of other infectious agents. The majority of the TAA identified so far belong to the first two categories and are normal self-structures expressed on tumor cells and certain normal tissue alike. These antigens dominate natural anti-tumor T cell responses in cancer patients and are most frequently used in cancer vaccines. Notwithstanding their being normal self, autoimmune reactions are rarely observed in cancer patients in the course of the disease or following therapeutic vaccination. Even when they occur, e.g. in the form of vitiligo in melanoma patients, they usually are restricted to isolated spots, often associated with tumor lesions. Thus, in response to cancer, the immune system seems to discriminate diseased from healthy tissue rather than self from non-self.

For cancer vaccines, TATE need to be selected for efficient induction of anti-tumor CTL responses. This implies that they should be as tumor-specific as possible, address, recruit and activate as many CTL as possible, be chosen to minimize the risk of selecting antigen-loss variants of the tumor cells and help to overcome tumor-associated immune suppression. Mimotopes of known TATE have been proposed to improve the binding of the peptides to their cognate MHC molecules, but also to enlarge the T cell repertoire to be mobilized against the tumor cells. This review will summarize the approaches to mimotope design, the new information on the specificities of T cells obtained from using mimotopes to explore T cell repertoires and specificity patterns, and clinical experiences from the application of mimotope vaccines for cancer immunotherapy.

2. Design of mimotopes for clinical application

In some cases where natural TATE were found to be inefficient in inducing T cell responses in *ex vivo* priming assays or in transgenic mice expressing HLA, exchange of amino acids involved in MHC binding was found to enhance efficacy of T cell induction [2–5]. Such improved epitopes were tested for induction of anti-tumor T cell responses in cancer patients. The results were mixed with some reports of improved T cell stimulation contrasted by others that show that T cells induced *in vivo* with such mimotopes efficiently lysed mimotope-pulsed cells but not necessarily tumor cells naturally expressing and presenting the original

epitope. Alternatively, mimotopes can be designed *de novo* using tumor-specific T cells to select active amino acids for the different sequence positions from combinatorial peptide libraries, thereby tailoring the mimotopes towards optimal stimulation of T cells with the capacity to lyse tumor cells presenting the natural counterpart of the mimotopes [6–16]. Such *de novo* generated mimotopes are designed around the basic structural features of MHC class I ligands [7]. These ligands are mostly nonapeptides that conform to MHC allele-specific sequence motifs, i.e. they have restricted variability of the amino acids at sequence positions that anchor the peptides into the peptide-binding grooves of the MHC molecules [17]. These motif amino acids vary with MHC molecule and allomorph. Combinatorial peptide libraries can be designed around these features to consist of mixtures of nonapeptides with either all sequence positions randomized or of singly defined sequence position in a randomized context [7,16]. The design of the peptide libraries is crucial for the development of mimotopes. Usually, the standard 19 proteinogenic amino acids, omitting cysteine, are used for the construction of the combinatorial peptide libraries. Cysteine can cause problems by crosslinking peptides because of oxidation and, therefore, is often excluded from the libraries. The peptide libraries are designed in scanning format, i.e. they have one defined sequence position, all other positions are randomized (Fig. 1), and the defined amino acid is varied through all sequence positions. Theoretically, every library which defines a fixed sequence position contains 19^n different peptides (n being the number of randomized sequence positions). The longer the peptides the more complex the mixtures are and the lower the likelihood of specific T cell responses to such libraries [18,19]. Cytotoxic T cell responses are best analyzed with octa- or nonapeptide libraries.

The recognition of peptides by T cells is restricted by specific MHC molecules, i.e. the peptides need to bind to

		sequence position				
		P1	P2	· · ·	Pn-1	Pn
scan position	P1	O	X		X	X
	P2	X	O		X	X
	·					
	·					
	Pn-1	X	X		O	X
	Pn	X	X		X	O

X: randomized sequence positions containing mixtures of the amino acids A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
O: defined sequence positions with one of the above amino acids

Fig. 1. Combinatorial peptide libraries. X denotes randomized sequence positions containing mixtures of the proteinogenic amino acids A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; O are defined sequence positions containing one of the before-listed proteinogenic amino acids in the indicated positions.

the MHC molecules in order to be recognized by the T cell. Every MHC allomorph has special requirements regarding the amino acids in the peptide that anchor it in the peptide-binding groove of the protein [17,20,21]. Usually, 2–3 sequence positions of the peptides serve as anchor residues for MHC binding. Given that until now more than 1800 different MHC class I molecules have been identified for the human population, the MHC-restricted peptide recognition by T cells significantly complicates the identification of active mimotopes. The anchor positions are often ill-defined by the combinatorial peptide library scans because peptide libraries that carry the anchor amino acids in the anchor positions contain a high fraction of competing peptides that bind efficiently to the MHC molecules without being recognized by the T cell. Therefore, in addition to the amino acids defined for the anchor positions by the combinatorial peptide library scans, the canonical motif amino acids as collated in the SYFPEITHI database may be considered for the design of mimotopes [17].

A complete set of nonapeptide libraries for mapping the specific sequence requirements for T cell mimotopes contains the completely randomized X₉ library and 171 OX₈ sublibraries for the 19 proteinogenic amino acids without cysteine, and nine sequence positions, theoretically 19⁹, i.e. about 323×10^9 different peptides (Fig. 1). The development of mimotopes using these combinatorial peptide libraries involves the following four steps (Fig. 2) [22].

- (1) Identification of the critical amino acids for the induction of tumor-specific T cell responses for every sequence position by testing the response of a suitable T cell clone to the combinatorial peptide libraries. This scan yields the potential mimotope amino acids.
- (2) Design and synthesis of potential mimotopes that include the different combinations of the active amino acids deduced from the library scans and, in cases where no amino acids were identified for the anchor positions, the canonical MHC-binding amino acids for the targeted MHC molecule.
- (3) Identification of the active mimotopes in a second T cell assay.
- (4) Validation of the mimotope as a T cell epitope with independent T cell lines generated by *ex vivo* priming and tested against mimotope-pulsed target cells and the tumor cells as controls for the specificities of the mimotope-induced T cells.

3. T cell specificities and T cell repertoires addressed by mimotopes

The complexity of the combinatorial peptide libraries with some 323×10^9 different peptides exceeds the complexity of the about 15×10^6 nonapeptide sequences that can be derived from the open reading frames of the human genome by more than 4 orders of magnitude. In effect, a large number of different mimotopes can be designed for every T cell clone [23]. Using the above-described combinatorial peptide library approach, the specificity profile of various T cell clones were mapped and found to be highly degenerate [9,23,24]. Because of this degeneracy, the number and quality of amino acids identified for the different sequence positions of the epitope sequences can vary largely between different T cell clones even in cases where they have the same epitope specificity. Different combinations of the active amino acids can yield mimotopes that differ substantially in their sequences and potencies to induce T cells. With different T clones different mimotopes or sets of mimotopes can be defined for the same natural epitope [23]. The sequences of the natural epitopes and their mimotopes can differ greatly. Usually, the mimotopes are more potent, sometimes by several orders of magnitude, than the natural epitopes because they are initially selected from combinatorial peptide libraries and tested for their capacity to induce T cell responses in the presence of large excesses of inactive peptides.

Complementary to the degeneracy of epitope/mimotope recognition by T cells, every mimotope defined for a given epitope can address a different T cell repertoire [23]. These different sets of T cells intersect around the natural epitope. This allows to recruit and activate T cells against the tumor cells that would not be activated by the natural epitope presented by the tumor cells. Thereby, mimotope vaccines can be designed to address broader T cell repertoires than vaccines containing the natural epitopes which increases the number of

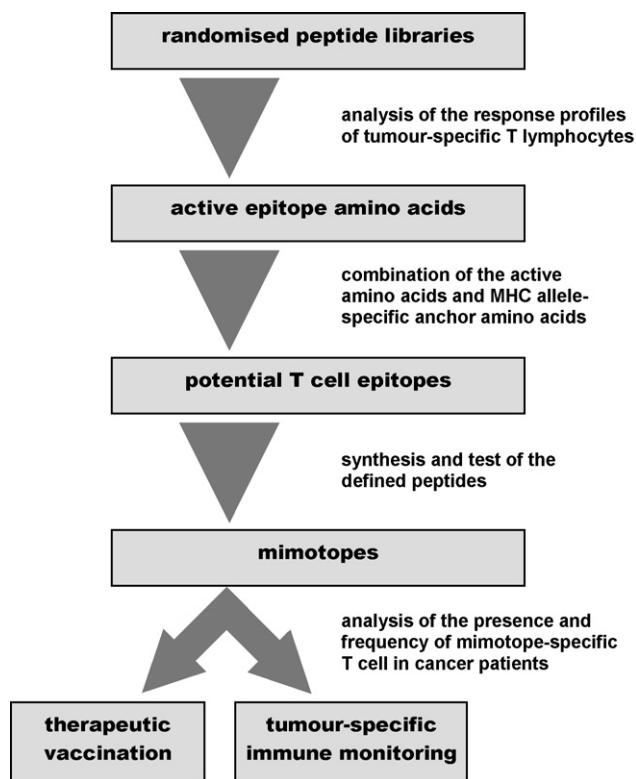


Fig. 2. The combinatorial peptide library approach to the identification of mimotopes for tumor-associated T cell epitopes.

T cells mobilized against the tumor [25,26]. In addition, the T cell repertoire that is recruited can be varied in the course of vaccination series to overcome or compensate suppression of tumor epitope-specific T cells in the tumor microenvironment [25–27].

Besides the possibility to vary the T cell repertoire utilized for anti-cancer immune intervention, the degeneracy of antigen recognition by T cells allows to select mimotopes that fulfill additional requirements such as optimal MHC binding or high resistance to proteolysis. Moreover, recent data suggest that different mimotopes can trigger different types of responses by the same T cell clone such as cytokine secretion versus cytolytic responses. Moreover, the combinatorial peptide library scans not only provide information on amino acids needed to design mimotopes that stimulate the T cells but also on amino acids that have the opposite effect and lead to T cell receptor antagonists that block T cell responses [7,8,11,13]. This opens the path for the development of antagonistic mimotopes for use in cases where pathogenic immune responses shall be suppressed. The observation that different mimotopes can induce different qualities of responses by the same T cell clones implies that the readout for the library scans has been chosen according to the desired quality of T cell response to be induced with the mimotope vaccine.

4. Mimotopes for cancer immunotherapy

As described above, the reasons for considering mimotopes as alternatives to natural tumor-associated T cell epitopes for the immune therapy of cancer are fourfold [26]. First, they are readily determined even in situations where no tumor material suitable for the determination of natural epitopes is available. Second, mimotopes are developed for most effective binding to the presenting MHC molecule and efficient stimulation of the cognate T cells. Third, they are expected to stimulate T cell repertoires that overlaps with, but differs from the repertoire addressed by the natural T cell epitopes and, thereby, can help to overcome tolerance or immunosuppression problems that might interfere with the therapeutic efficacy of natural epitopes. Fourth, they can be designed to induce specific qualities of T cell responses best suited for therapy.

5. Clinical tests of the mimotope cancer vaccine concept

Using the above-described approach, we developed mimotopes for a CTL clone specific for the tumor cells of a patient with cutaneous T cell lymphoma (CTCL) [25]. These mimotopes, nonapeptides with the sequences PVKTKDIKL and PVKTYDIKL, were designed according to the HLA restriction of the original CTL clone for presentation by HLA-B8. PVKTKDIKL adheres exactly to the HLA-B8 binding motive with positively charged amino acids at positions 3 and 5, and

an aliphatic amino acid at position 9. PVKTYDIKL diverts from this motive by a tyrosine at position 5 which was most potent at this position in the positional scan. CD8⁺ T lymphocytes specific for these mimotopes had been found in the peripheral blood of more than 90% of HLA B8-expressing patients suffering from different forms of CTCL with frequencies among the CD8⁺ T cells ranging between 0.03 and 1.42%. The mimotopes, thus, appear to mimic a TATE and its source TAA that is widely shared by the tumor cells in different CTCL variants and individual patients. In contrast, only in a few exceptional cases mimotope-specific T cells were detected in the peripheral blood of healthy controls.

In an initial clinical proof-of-principle test, two patients were treated with a vaccine composed of the above two mimotopes admixed with recall antigens tested positive for the respective patients in skin hypersensitivity tests. The recall antigens served hereby to stimulate the help by CD4⁺ T cells required for the induction of tumor-specific CTL and were chosen because of the already preexisting high-level T cell immunity against these antigens [26,28,29]. The skin test performed to identify the most suitable helper antigens for the respective patients reactivated this preexisting immunity and is meant to enhance the level of T cell help for induction of the mimotope-specific T cells. The vaccines were injected intradermally at sites not affected by tumor lesions in 4-week intervals. The clinical and the immunological responses were monitored before and once a day for the first week after each vaccination, and once a week thereafter. Mimotope-specific CD8⁺ T cells were visualized by intracellular staining for interferon γ following incubation with the mimotopes, and detected and enumerated by flow cytometry.

Both patients experienced fulminant inflammatory reactions after the first vaccination with subsequent disappearance of all tumor lesions [26]. The complete responses were confirmed by histopathological and immunohistochemical inspections of biopsies taken from skin areas affected by the tumor before vaccination. The complete clinical responses lasted for 3 and 10 months, respectively, after which time the patients received conventional therapies. In the peripheral blood of both patients increased frequencies of mimotope-specific CD8⁺ T cells were detected after vaccination. The frequencies of these cells rose from undetectable to 0.21% in the first and 0.52% in the second patient corresponding to between 2100 and 5000 specific T cells per ml, or total numbers in the peripheral blood of up to 5.5×10^6 and 13×10^6 in the two patients, respectively. It is expected that these are low estimates and that the majority of mimotope-specific cells are sequestered in other compartments such as lymphatic organs and tumor lesions. In any case, the massive inflammatory response affecting all tumor lesions of the patients and the complete remissions testify to the capacity of the mimotope-induced T cells to infiltrate all tumor lesions and to destroy the tumor cells. The tumor-specificity of the mimotope-induced T cells was confirmed by isolating cells responding to the mimotopes by interferon γ capture and magnetic bead separation, expanding them in culture by repeated stimulation

with the respective mimotopes and testing their capacity to lyse tumor cells from the same patient.

This initial clinical test proves that the mimotopes can induce specific T cell responses in cancer patients, that these T cells are specific for the tumor cells and that they have the capacity to attack and lyse the tumor cells in the skin lesions at various sites far away from the sites where the vaccines were injected. The natural epitope corresponding to the mimotopes and the source protein of this epitope are not known yet. However, the high proportion of patients having elevated frequencies of mimotope-specific T cells in their peripheral blood indicate that the source protein of the natural counterpart of the mimotopes is a TAA that is widely shared among CTCL patients [25]. Both patients relapsed after the complete responses. These relapses were not due to exhaustion of the tumor/mimotope-specific T cells nor to antigen loss by the tumor cells as, first, mimotope/tumor-specific CD8⁺ T cells could still be detected in the peripheral blood of the patients at late stages of the disease and, second, tumor cells circulating at this same late stage were still susceptible to lysis by the CTL [26]. Failure of the immunotherapy in these cases is likely due to disease-related immune suppression. These observations indicate, that the combinatorial peptide library approach can lead to TATE and TAA that are relevant for the tumor cells and sensitive targets for therapeutic vaccination, thereby validating the mimotope concept for cancer immunotherapy.

Since the source protein corresponding to the mimotopes is not known, vaccine-induced autoimmunity is a serious concern. The patients were, therefore, extensively monitored for indications of autoimmune reactivity without that any sign of such adverse reactions was found. Other side effects were restricted to inflammatory reactions at the vaccine injection sites and tumor lesions. This low toxicity of mimotope vaccination is common to all therapeutic vaccination strategies tested for cancer to date. Notwithstanding the absence of adverse effects in the initial mimotope vaccination test, possible autoimmune reactions need to be monitored also in future trials. The failure of the therapeutic vaccination after initial complete responses may be caused by exhaustion of the tumor-specific T cell responses or by tumor-associated immune suppression. The impact of exhausting the T cells activated against the tumor cells after repeated booster vaccination may be overcome by changing the mimotopes in the course of extended treatment regimens [26,27]. Immunosuppression, on the other hand, is a problem that limits the efficacy of all immune intervention strategies and needs to be addressed by identifying the molecular mechanisms of tumor-related immunosuppression and blocking the immune-suppressive factors [30]. Such accompanying measures have been tested in clinical vaccination trials but, as yet, are hampered by frequent induction of autoimmune effects. Future developments need to identify measures against tumor-specific immune-suppression mechanisms that can be combined with therapeutic vaccination, e.g. with mimotopes.

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