*Annals of Oncology* 7: 339–342, 1996.


# Review

## Recognition of tumor-associated antigens by T-lymphocytes: Perspectives for peptide-based vaccines

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**Key words**: cancer vaccines, cytolytic T-lymphocytes, tumor antigens

### Introduction

Over the last 10 years, studies of T-cell-mediated responses *in vitro* have clearly demonstrated that some cancer patients have (mainly but not exclusively) $CD8^+$ cytolytic T-lymphocytes (CTL) that can recognize specifically autologous tumor cells [1]. Until 1991, however, no CTL-defined tumor antigen was characterized in molecular terms in humans. With the development of new approaches based on recent advances in our basic understanding of the processes involved in antigen recognition by T-cells [2, 3] the past 5 years have witnessed the molecular definition of more than a dozen human tumor antigens recognized by $CD8^+$ T-cells.

While studies in murine tumor model systems suggested that peptides derived from mutated gene products would be the primary candidates for tumor antigens recognized by CTL [4–7], most of the human tumor-associated antigens defined thus far are derived from normal (non-mutated) proteins. Since these antigens have been identified by virtue of their recognition by autologous CTL clones, it is now evident that the field of tumor immunology is more closely related to autoimmunity (or immune recognition of self proteins) than to immune responses to foreign antigens. In this review, we first discuss the strategies used to identify tumor-associated, CTL-defined peptide antigens. We then describe some of the antigens that are currently considered as potential targets for peptide-based vaccines. Finally, we briefly summarize the first attempts to elicit specific CTL responses in cancer patients with such vaccines.

### Strategies to identify tumor-associated antigens

$CD8^+$ CTL recognize antigenic peptides produced by the degradation of intracellular proteins that are transported to the cell surface in association with major histocompatibility complex (MHC) class I molecules (i.e., HLA-A, -B and -C in humans). In normal cells as well as in tumor cells, most of the MHC class I molecules expressed at the cell surface are associated with peptides produced by the degradation of normal proteins. Moreover, there is a large number ($\geqslant$10.000) of different peptides associated with MHC class I molecules expressed by a given cell. Finally, the set of different peptides presented at the cell surface varies between MHC class I isoforms (or alleles), e.g., HLA-A1 and HLA-A2 [8]. Because of the extreme diversity of peptides naturally associated with MHC class I molecules, the identification of the antigenic peptides naturally recognized by tumor-specific CTL is not a trivial task, and three new methods have been developed for that purpose.

The first method is based on a genetic approach whereby the gene encoding the tumor antigen is identified by testing appropriate target cells transfected with recombinant DNA libraries or cDNA libraries prepared from tumor cells for recognition by autologous tumor-specific CTL clones [9–11]. Once the gene has been identified, the region encoding the antigenic peptide can be narrowed down by transfecting gene fragments. The amino acid sequence deduced from the nucleotide sequence of this region is used to produce synthetic oligopeptides which are then tested in a target cell sensitization assay for recognition by the original tumor-specific CTL clones. The rationale for such an approach is based on the demonstration that synthetic peptides can mimic endogenously derived peptides in appropriate CTL assays *in vitro*.

The same rationale has been applied in the development of a second method based on a biochemical approach in which peptides associated with MHC class I molecules expressed by tumor cells are extracted and fractionated by high pressure liquid chromatography [12, 13]. The peptide fractions are then tested in a target cell sensitization assay for recognition by tumor specific CTL clones. After several steps of fractionation, the sequences of the peptides recognized by CTL are obtained by the use of tandem mass spectrometry [14].

While these two approaches rely on the availability

of tumor-specific CTL clones derived from T-cell populations of cancer patients, a third method is based on the induction of CTL responses *in vitro* using normal peripheral blood lymphocytes stimulated with synthetic peptides corresponding to linear amino acid sequences of a known tumor-associated protein and which are selected on the basis of their ability to bind to a particular MHC class I isoform. Once peptide-specific CTL clones are obtained, they are used to assess whether the same peptides are presented at the surface of tumor cells upon endogenous processing of the tumor-associated protein [15–18].

## CTL-defined tumor antigens

The first human tumor antigen to be defined in molecular terms was identified using the genetic approach mentioned above [9]. Because the CTL clone used to identify the antigen was directed against autologous melanoma cells, the gene encoding the antigen was called *MAGE-1* ('M' for melanoma and 'AGE' for antigen). However, the gene is not only expressed in some melanomas, but also in a significant proportion of other tumors, including non-small cell lung carcinomas, mammary carcinomas, head and neck squamous cell carcinomas and bladder carcinomas [11, 19–21]. In contrast, *MAGE-1* is silent in normal tissues, except testis. The CTL-defined antigens identified thus far are two distinct nonapeptides which associate with HLA-A1 and HLA-Cw16 molecules, respectively [22, 23]. *MAGE-1* belongs to a family of 12 genes located on chromosome X [24]. Among this family, *MAGE-3* also encodes CTL-defined antigens, which have been identified as nonapeptides or decapeptides associated with HLA-A1, HLA-A2 or HLA-B44, respectively [15–18, 25]. It should be stressed that the sequences of genes *MAGE-1* and *MAGE-3* are identical in tumor cells and normal cells. Interestingly, the frequency of *MAGE-1* and *MAGE-3* expression in malignant melanoma is significantly higher in metastatic lesions than in primary lesions [26]. Both genes are silent in melanocytes, in benign and in dysplasic nevi as well as in *in situ* melanomas. In primary melanomas, the frequency of expression of both genes increases with tumor thickness [26]. Similarly, a higher frequency of *MAGE* gene expression in invasive than in superficial lesions is found in primary transitional cell carcinomas of the bladder [21]. As the function(s) of *MAGE-1* and *MAGE-3* gene products is unknonw, it is unclear whether they play any role in tumor progression. Moreover, further work is needed to ascertain whether *MAGE-1* and/or *MAGE-3* expression is homogeneous in a given tumor. It should also be kept in mind that there is presently no way to quantitate the amount of antigenic peptides derived from the *MAGE-1* and *MAGE-3* proteins that are displayed on the surface of tumor cells. Obviously, these limitations in our knowledge should be taken into account in the future development of peptide-based vaccines.

Other antigens recognized by melanoma-specific CTL clones have been identified by the genetic approach. Since the genes encoding these antigens, like the *MAGE* genes, are silent in normal tissues, except testis, and expressed in different tumor types, they have been designated *BAGE* and *GAGE* [27, 28]. The antigenic peptides identified thus far are associated with HLA-Cw16 and HLA-Cw6 molecules, respectively. Because these two HLA alleles are relatively rare in the Caucasian population, the usefulness of these antigenic peptides for cancer vaccines has yet to be defined.

In contrast to the antigens described above, another category of CTL-defined tumor antigens is lineage-specific, i.e., the genes involved are expressed in both normal and cancer cells of the same histological type. The prototypes of such antigens are the antigenic peptides derived from proteins, such as tyrosinase [29–32], gp100 [14, 33] gp75 [34] and Melan-A/MART-1 [35, 36] that are expressed in cells of the melanocytic lineage (i.e., melanocytes and melanomas). Interestingly, the majority of the peptides defined thus far are associated with HLA-A2, which is expressed in about 50% of the Caucasian population. Moreover, the great majority of malignant melanomas express the genes encoding tyrosinase and Melan-A, and the expression of the proteins appears homogeneous within individual tumors. Altogether, these features make such antigenic peptides good candidates for peptide-based vaccines in melanoma. Obviously, there is a risk of generating CTL responses directed against both melanocytes and melanoma cells with such vaccines. In this context, it is noteworthy that the occurrence of vitiligo (presumably as a result of T-cell mediated elimination of melanocytes) in melanoma patients is associated with prolonged survival [37, 38]. It thus appears justified to include melanocyte lineage-specific peptides defined by CTL in the construction of melanoma vaccines.

As stated previously, peptides derived from cellular oncogene products were considered attractive candidates for human CTL-defined tumor antigens since the corresponding proteins are often mutated in tumor cells. Indeed, peptides derived from mutated forms of *p21*ras or *p53* have been identified using the approach based on *in vitro* CTL responses induced by selected synthetic peptides [39–41]. With few exceptions, however, the CTL clones obtained under these conditions did not recognize tumor cells expressing the relevant mutated protein, thus suggesting that no (or too few) endogenously derived peptides corresponding to the synthetic peptides used for CTL induction *in vitro* were present at the tumor cell surface.

In contrast, there is evidence that CTL-defined antigens can be derived from the HER-2/neu oncogene product [42, 43]. Interestingly, the identified peptides correspond to the normal *HER-2/neu* sequence. Since the HER-2/neu product is often overexpressed in tumor cells, it is likely that quantitative, rather than qualitative, differences in endogenously produced peptides

are responsible for the specific CTL recognition of tumor cells as compared to normal cells. Tumor types that have been shown to be recognized by HER-2/neu peptide-specific CTL include ovarian, breast and non-small cell lung carcinomas [44, 45].

From this brief survey of CTL-defined tumor antigens, which is by no means comprehensive, it should be evident that a large number of antigenic peptides have recently been identified which are potential candidates for peptide-based cancer vaccines. Indeed, this information is already being used to design phase I clinical trials based on the use of single tumor-associated peptide antigens.

## Clinical prospects

The molecular characterization of tumor antigens potentially able to be recognized by CTL opens up new strategies for attempts at active immunization in human cancer. In contrast to the various types of cancer vaccines used in the past, there is now the possibility to use tailor-made vaccines that only contain relevant tumor antigens. The first step consists of determining for individual cancer patients the panel of tumor-associated peptides presumably expressed by their tumor cells. This can be done by assessing the expression of the relevant genes or gene products in a small tumor sample. In combination with the determination of the MHC class I alleles expressed in the patient, this information is used to select the relevant synthetic peptides to be included in a vaccine preparation.

For practical reasons, the current phase I clinical trials based on the use of CTL-defined peptides have involved administration of peptide alone. While some positive responses have been reported [46, 47], it is evident that peptide antigens demonstrate excellent safety but also weak immunogenicity. Thus, the need for improving the immunogenicity of peptides has renewed interest in adjuvants. Evaluation of these new vaccine strategies is still at a very early stage, and it is likely that a relatively large number of phase I clinical trials will be necessary to evaluate the various procedures that are being proposed. Moreover, careful monitoring of specific CTL responses elicited by peptide-based vaccines is necessary to assess the efficacy of the immunization procedure in individual patients. In this respect, it should be kept in mind that little is known on the ability of adjuvants to augment CTL responses to peptide antigen in humans. Because alum adjuvants increase antibody responses rather than T-cell responses, several new adjuvants are currently under evaluation in clinical trials [48]. The use of appropriate adjuvants in peptide-based cancer vaccines may be particularly crucial since there is increasing evidence for alterations in the immune responsiveness of cancer patients [49].

An alternative approach for the generation of CTL *in vivo* is the administration of autologous dendritic cells loaded with the corresponding peptide antigens. Dendritic cells are often referred to as 'professional' antigen-presenting cells since their ability to initiate antigen-specific T-cell responses is superior to that of other antigen-presenting cells [50]. Based on positive results obtained in murine tumor model systems [51], attempts to use tumor peptide-loaded dendritic cells have been initiated [52]. As mentioned above, the validity of such an approach will only be established in carefully planned clinical trials.

In summary, the recent identification of molecularly defined tumor antigens that can be mimicked by synthetic peptides provides new vaccine possibilities in cancer. Vaccine construction can be adjusted to the antigenic profile exhibited by individual tumors. Careful investigation of immunized patients is required in order to develop effective cancer vaccines.

## Acknowledgements

We thank J. Duc, A. Zoppi and M. Overloop for secretarial assistance.

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Received 29 March 1996; accepted 2 April 1996.

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