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INMUNOTHERAPY OF MELANOMA: PEPTIDE MIMICS OF A HUMAN HIGH MOLECULAR WEIGHT-MELANOMA ASSOCIATED ANTIGEN

XINHUI WANG, WEI LUO, SOLDANO FERRONE

Department of Immunology, Roswell Park Cancer Institute, Buffalo NY, USA

Abstract The realization that tumor cells utilize multiple mechanisms to escape from immune recognition and destruction has stimulated interest in developing and applying immunotherapeutic strategies which target both humoral and cellular immunity to malignant cells. As a result, the tumor-associated antigens (TAA) used as targets have to be expressed on the cell surface membrane of malignant cells. Furthermore, since most of the TAA used for active specific immunotherapy are self-antigens, a challenge facing tumor immunologists is to develop strategies which are effective in breaking tolerance to self-antigens. This chapter describes one strategy which relies on the use of peptide mimics of the human high molecular weight-melanoma associated antigen (HMW-MAA) as immunogens to implement active specific immunotherapy in patients with malignant melanoma. These mimics, which are isolated from phage display peptide libraries by panning with anti-HMW-MAA monoclonal antibodies, are expected to induce both humoral and cellular anti-HMW-MAA immunity.

Key words: melanoma, active specific immunotherapy, peptide mimics, melanoma associated antigens

Resumen ***Inmunoterapia en melanoma. Simulador peptídico del antígeno humano de alto peso molecular asociado a melanoma.*** Al comprender que las células tumorales utilizan múltiples mecanismos para evadir el reconocimiento y destrucción inmunológica, se ha despertado interés en el desarrollo y aplicación de estrategias inmunoterapéuticas que tienen como blanco la inmunidad humoral y celular contra las células malignas. En consecuencia, los antígenos asociados a tumor (TAA) usados como blanco tienen que expresarse sobre la superficie de las células malignas. Además dado que la mayoría de los TAA usados para inmunoterapia activa específica son auto-antígenos, el desafío para los inmunólogos es desarrollar estrategias que sean efectivas para romper la tolerancia de estos auto-antígenos. Este trabajo describe una estrategia que se basa en el uso de un péptido que simula el antígeno humano de alto peso molecular asociado al melanoma (HMW-MAA) como inmunógeno para implementar una inmunoterapia activa específica en pacientes con melanoma maligno. Estos péptidos aislados de fagos se detectan en librerías peptídicas a través de una selección con anticuerpos monoclonales anti -HMW-MAA los cuales potencialmente inducirían inmunidad humoral y celular anti -HMW-MAA.

The identification and molecular characterization of human tumor associated antigens (TAA) during the last few years[1-4] have provided well defined moieties to implement active specific immunotherapy in patients with malignant diseases[5, 6]. For several years the emphasis has been on T cell-defined TAA because of the general belief that T cells are the major, if not the only players in the control of tumor growth and because of the disappointing results of the early clinical trials relying on anti-TAA antibodies[7]. The realization that the multiple mechanisms utilized by malignant cells to escape from T cell recognition represent a major limitation in the successful application of T cell-based immunotherapy of malignant diseases[8] has rekindled interest in the utilization of anti-TAA antibodies, by themselves or in combination with $CD4^+$ and/or $CD8^+$ T cells, to control tumor growth. This trend has been strengthened by the association between induction of anti-TAA antibodies in patients with malignant diseases and improved prognosis[9-11] and by the recent favourable results of passive immunotherapy of malignant diseases with anti-TAA antibodies by themselves or in combination with chemotherapy[12-14].

The large majority of TAA identified in malignant cells with T cells or with antibodies have been found to be self – antigens[15, 16] which are expressed in larger amounts in malignant cells than in their normal counterparts, most likely because of abnormalities in gene regulation associated with the transformation process. Therefore a challenge facing tumor immunologists in applying active specific immunotherapy of malignant diseases is to develop and utilize approaches which are effective in breaking tolerance to self-antigens.

Postal address: Dr. Soldano Ferrone, Department of Immunology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, USA
Fax: (1-716) 845-7613 e-mail: soldano.ferrone@roswellpark.org

Among the many approaches which are being tested we have selected the one relying on the utilization of mimics of TAA as immunogens. The rationale for our choice derives from the results of our previous clinical trials in malignant melanoma utilizing mouse anti-idiotypic monoclonal antibodies which mimic the high molecular weight-melanoma associated antigen (HMW-MAA)[9, 17]. We found that anti-idiotypic monoclonal antibodies were more effective than the original TAA in breaking tolerance to a self-antigen. Anti-idiotypic antibodies elicited anti-HMW-MAA antibodies in more than 50% of the immunized patients, while the HMW-MAA was not immunogenic. This finding is likely to reflect the deletion, during the establishment of self-identity, of B cell clones that recognize the HMW-MAA with high affinity. In contrast the immunogenicity of the corresponding anti-idiotypic antibodies is likely to reflect their ability to stimulate clones which have not been deleted during the establishment of self-identity, since they secrete antibodies reacting with the corresponding antigen with an affinity below the threshold required for their deletion. We have selected the HMW-MAA as a target of immunotherapy because of its high frequency of expression in patients with melanoma[18, 19], its high expression by melanoma cells with limited intra- and inter-lesional heterogeneity[19, 20], its restricted distribution in normal tissues[18, 19] and its suggested role in the metastatic potential of melanoma cells[21, 22]. Furthermore the expression of HMW-MAA by pericytes[23] suggests that the effect of anti-HMW-MAA immunity on melanoma lesions may be mediated not only by a direct interaction with melanoma cells, but also by disturbing the blood supply.

The mimics of HMW-MAA we plan to use as immunogens are represented by peptides we have isolated by panning phage display peptide libraries with mouse anti-HMW-MAA monoclonal antibodies and with human anti-HMW-MAA single chain Fv fragments. Analysis of the isolated peptides has shown that the large majority of them do not display a significant homology in their sequence with the published amino acid sequence of the HMW-MAA[24]. Furthermore the isolated peptides have distinct sequences. Most of the peptides react only with the antibody used for their isolation and do not crossreact even with antibodies which display a high degree of homology in the amino acid sequence of the variable regions of their heavy and light chains with those of the antibodies used for their isolation. Only the peptides isolated from the phage display peptide library X15[25] with the mouse monoclonal antibodies 149.53 and 225.28 display homology with the amino acid sequence of HMW-MAA. As shown in Table 1, the sequences of the peptides isolated with the monoclonal antibodies 149.53 and 225.28 are identical to that of the HMW-MAA at positions 1846-1850 and 1852 and at positions 1457-1460, respectively. It is noteworthy that the monoclonal antibodies 149.53 and 225.28 crossreact with the rat antigen NG2, a chondroitin sulfate proteoglycan isolated from a chemically induced rat neuronal tumor[26]. The human HMW-MAA displays an approximately 80% homology with the rat NG2 antigen in its amino acid sequence[24, 27]. The aminoacids shared by the peptides isolated with the monoclonal antibodies 149.53 and 225.28 with the HMW-MAA are also present in the NG2 antigen[27], therefore strengthening the possibility that these amino acids play an important role in the expression of the determinants recognized by the two monoclonal antibodies. Interestingly, both monoclonal antibodies are; less reactive with the rat NG2 antigen than with the human HMW-antigen restricted, HMW-MAA specific cytotoxic T lymphocytes in addition to anti-HMW-MAA antibodies, ii) they eliminate the induction of antibodies to constant and variable regions of mouse anti-idiotypic monoclonal antibodies and iii) they facilitate the development of immunogens resulting from the fusion of peptide(s) with cytokines which are likely to display an increased immunogenicity. Lastly, from a practical view point it is easier and less expensive to prepare synthetic peptides to be used as immunogens in clinical trials than mouse anti-idiotypic monoclonal antibodies.

TABLE 1.– *Homology with the human HMW-MAA and with the rat NG2 antigen of peptides isolated from the phage display peptide library X15 by panning with the anti-HMW-MAA monoclonal antibodies 149.53 and 225.28*

Monoclonal antibody	Homology with HMW-MAA and NG2 antigen of peptides isolated with monoclonal antibodies	
149.53	Peptide	EELHPP**GSRAP**S I**R**K
	HMW-MAA	PLRLTR**GSRAP**IS**R**A
	NG2	P LRITR**GSRAP**VS**R**A
225.28	peptide	TQYTRTDPWG**LEPP**K
	HMW-MAA	PT CLGLSLQ V**LEPP**Q
	NG2	GTCPGLVLQ V**LEPP**Q

Amino acids which are identical in the isolated peptides and in the antigen analyzed are bolded

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Indeed a great deal of industrious work is being done on cancer ... but someone should have another bright idea.

De hecho se está haciendo muchísimo trabajo de peso en cáncer ... pero alguien tendría que venir con otra brillante idea.

Rudolf Virchow (1821-1902)