GENES CODING FOR MELANOMA ANTIGENS RECOGNISED BY CYTOLYTIC T LYMPHOCYTES

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SUMMARY

It is now well established that human melanoma cells express antigens that are recognised by cytolytic T lymphocytes derived from the tumour-bearing patient. The molecular definition of these antigens is progressing at an accelerated pace. The currently characterised melanoma antigens can be classified into three categories: differentiation antigens, antigens encoded by genes that are specifically expressed in tumours, and antigens encoded by mutated genes. Several of these antigens are sufficiently tumour-specific to qualify them as candidate anti-cancer vaccines in melanoma patients.

The prospects for cancer immunotherapy are based mainly on the assumption that cancer cells express specific antigens recognised by T lymphocytes, as these cells have been shown to mediate tumour rejection in animal models.[1]

For human melanoma, it is often possible to obtain autologous anti-tumour cytolytic T lymphocytes (CTL) by co-cultivating irradiated tumour cells with blood lymphocytes of the tumour-bearing patient.[2] This procedure is called the Mixed Lymphocyte-Tumour cell Culture (MLTC). Anti-tumour CTL clones with high activity and specificity have been obtained.[3,4] Detailed analyses of these CTL clones demonstrated that melanoma cells express multiple antigens.[4–6]

Most anti-melanoma CTL are $CD8^+$ T lymphocytes. Antigens recognised by $CD8^+$ CTL are small peptides inserted in a groove of the class I Major Histocompatibility Complex molecules, HLA-A, -B or -C (Fig. 1). The peptides are produced inside the same cells, mostly by degradation of cellular proteins, and are transported in the endoplasmic reticulum where they combine with the heavy chain of HLA class I molecules. Following association with β_2-microglobulin, the complexes migrate to the cell surface where they can be recognised by the receptor of the CTL.

The first experimental approaches that led to the molecular identification of antigens recognised by anti-tumour CTL were based on the transfection of recombinant DNA libraries.[7] More recently, we resorted to another genetic approach based on the transient transfection of cDNA libraries into COS cells.[8] Once the gene coding for a tumour antigen has been identified, the region encoding the antigenic peptide can be narrowed down by transfecting gene fragments. Synthetic peptides can then be tested for recognition by the CTL.

During the last few years we have characterised several genes encoding human melanoma antigens recognised by CTL. The antigens encoded by these genes fall into three categories: differentiation antigens, antigens encoded by genes that are specifically expressed in tumours, and antigens encoded by mutated genes.

MELANOCYTIC DIFFERENTIATION ANTIGENS

Several groups have cloned from autologous MLTC CTL that lysed not only the stimulator melanoma cells but also allogeneic melanoma cells.[9–11] Some of these CTL also recognised allogeneic melanocytes, suggesting that the target antigens were expressed not only by melanoma cells but also by normal cells of the melanocytic lineage.[12]

The first melanoma shared antigens that were characterised proved to be encoded by the tyrosinase

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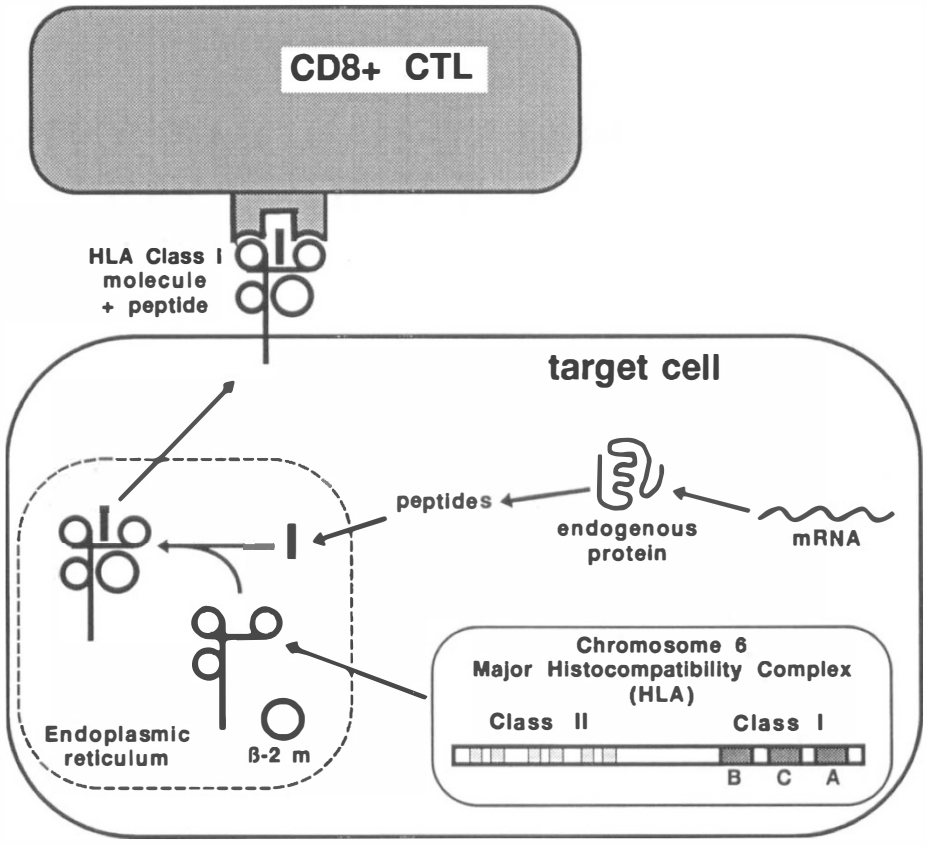

Fig. 1. *Mechanism of presentation of an antigen to CD8$^+$ cytolytic T lymphocytes.* β-2 *m,* β_2*-microglobulin.*

gene.[13] This enzyme converts tyrosine into dihydroxyphenylalanine (DOPA), the precursor of melanin. The tyrosinase gene is expressed in normal melanocytes but not in any other normal tissue. Consistently, tyrosinase is expressed in virtually all melanoma samples, but in no other types of tumours. We have so far identified three antigenic peptides encoded by the tyrosinase gene that are recognised by specific CTL clones: two are presented by HLA-A2 class I molecules[14] and one by HLA-B44.[15]

Another gene was cloned that encodes a shared melanoma antigen presented by HLA-A2.[8] This new gene presents a pattern of expression similar to that of the tyrosinase gene. Owing to its melanocytic expression, the gene was named Melan-A. Another group identified the same cDNA as coding for an antigen recognised by an HLA-A2-restricted CTL line derived from the lymphocytes infiltrating a melanoma, and named it MART-1.[16]

Pmel17 and gp100 are two melanocyte-specific glycoproteins originating from the same gene via alternative splicing: gp100 contains 641 amino acids, and Pmel17 has an additional stretch of 7 amino acids inserted in position 567.[17,18] Different groups have identified antigenic peptides derived from the Pmel17/gp100 proteins that are presented at the surface of melanoma cells by HLA-A2 molecules.[19,20] They used either the genetic approach to isolate the cDNA encoding the target antigen,[21] or a biochemical approach to fish the antigenic peptide eluted from HLA-A2 molecules extracted from melanoma cells.[20] Interestingly, when we performed a MLTC with tumour cells derived from an HLA-A2 melanoma patient whose tumour expressed high levels of melanocyte-specific genes, the majority of the CTL clones that we obtained (40 of 46) were directed against one of the above-mentioned Pmel17/gp100 antigens.[22] This suggests that this antigen can elicit a strong T-cell response.

TUMOUR-SPECIFIC ANTIGENS

The first human tumour-specific antigen that was identified is antigen MZ2-E, which is expressed by melanoma cell line MZ2-MEL.[23] The gene encoding this antigen was named *MAGE-1*, for *M*elanoma *A*nti*GE*n. The sequence of the gene shows no significant identity with known human genes. Gene *MAGE-1* contains three exons. An open reading frame coding for a protein of 309 amino acids is located in the third exon. Gene *MAGE-1* is a member of a group of at least 12 closely related genes.[24] All *MAGE* genes are located on chromosome X, in Xq28.[25] More recently, another cluster of

Table I. Expression of *MAGE, BAGE* or *GAGE* genes in tumour samples

	% of positive tumour samples			
	MAGE-1	*MAGE-3*	*BAGE*	*GAGE*
Melanomas	36	64	22	35
Head and neck carcinoma	25	48	8	28
Lung (NSCLC)	36	31	7	30
Bladder carcinomas	19	33	14	12
Breast carcinomas	19	11	10	13
Sarcomas	10	19	8	30
Prostatic carcinomas	15	15	0	15
Colorectal carcinomas	0	16	0	0
Renal carcinomas	0	0	0	0
Leukaemias/lymphomas	0	0	0	0

Expression was assessed by PCR amplification of reverse-transcribed RNA extracted from tumour samples frozen immediately after surgery.

MAGE-related genes has been identified in Xp21.[26] Only *MAGE-1* codes for antigen MZ2-E, which is presented by HLA-A1 class I molecules.[27] However, another tumour antigen expressed on melanoma cell line MZ2-MEL was later found to be encoded by *MAGE-3*.[28]

The expression of the *MAGE* genes was evaluated by reverse transcription and PCR amplification of RNA extracted from normal tissues and from tumours. Several sets of primers were chosen that are specific for each *MAGE* gene. The expression of the *MAGE* genes was absent in all the normal tissue samples, with the exception of testis and, for *MAGE-3* and *MAGE-4*, placenta.[24,29] The expression of the *MAGE* genes in testis is probably restricted to the germline cells.[30] Genes *MAGE-1,-2,-3,-4,-6* and *-12* are also expressed in tumours of different histological types (Table I). Surprisingly, in contrast to cutaneous melanomas, uveal melanomas rarely express *MAGE* genes.[31] *MAGE-1, -2* and *-3* were shown to be more often expressed by metastatic melanomas than by primary tumours.[32]

To understand the basis of the tumour-specific expression of the *MAGE* genes, we have studied the *MAGE-1* promoter. Three regulatory elements account for most of the *MAGE-1* promoter activity: one binds to Sp1 factors, and the two others bind to factors of the Ets family of transcription factors.[33] The *MAGE-1* promoter exhibits transcriptional activity in tumour cell lines that do not express *MAGE-1*, indicating that these cells contain transcription factors capable of inducing *MAGE-1* promoter activity. One could speculate on the existence of a sequence located outside the *MAGE-1* promoter, such as a silencer, involved in the cell-specific expression of *MAGE-1*. However, recent data suggest that DNA methylation plays an important role in the regulation of *MAGE-1* transcription: (i) there is a striking correlation between *MAGE-1* expression and demethylation of its promoter; (ii) treatment with the demethylating agent azadeoxycytidine activates *MAGE-1* expression in normal cells and in tumour cell lines that do not express the gene; (iii) the expression of *MAGE-1* occurs in tumour cell lines showing an important decrease in the overall DNA methylation level.[34] Taken together, these observations indicate that the expression of *MAGE-1* in cancer cells is due to the demethylation of the promoter, and that this is a consequence of a genome-wide demethylation process. Genome-wide demethylation is known to occur in tumour cells and to be more pronounced in metastatic[35–37] than in primary tumours. It is also known to occur in the male germline cells during spermatogenesis.[38,39]

Another tumour antigen expressed by melanoma cell line MZ2-MEL was found to be encoded by a new gene, named *BAGE*.[40] This gene codes for a putative protein of 43 amino acids and seems to belong to a family of several genes. Like the *MAGE* genes, *BAGE* is not expressed in normal tissues except testis, and is expressed in several tumour types (Table I). Yet another MZ2-MEL tumour antigen was characterised, and led to the identification of a new family of genes named *GAGE*.[41] Two members of this family encode the antigenic peptide presented by HLA-Cw6. Again, the *GAGE* genes are silent in normal tissues except testis, and are expressed in different tumour types (Table I).

Antigen NA-17 is recognised on another melanoma by tumour-infiltrating lymphocytes. This antigen was found to be encoded by an abnormal mRNA resulting from the activation of an alternative promoter located in the intron of the *N*-acetylglucosaminyltransferase V (GnT-V) gene.[42] Whereas the normal GnT-V mRNA is expressed ubiquitously, the mRNA encoding the NA-17 antigen is not expressed in normal cells but is found, among tumours, in about 50% of melanomas.

ANTIGENS ENCODED BY MUTATED GENES

Several years ago, the study of immunogenic variants of mouse tumours obtained by mutagenesis showed

that point mutation in genes expressed ubiquitously could create antigenic epitopes recognised by CTL.[7,43]

In human melanoma LB33-MEL, an antigen recognised by autologous CTL was found to result from a point mutation in a previously unknown ubiquitously expressed gene.[44] The mutation was observed only in the melanoma cells of patient LB33. Surprisingly, the mutation is located in an intronic sequence, indicating that the antigenic peptide derives from the translation product of an incompletely spliced mRNA.

Another very interesting example is the point mutation of cyclin-dependent kinase 4, which produces an antigenic peptide that is presented by HLA-A2 class I molecules at the surface of SK29-MEL melanoma cells.[45] This mutation alters the regulation of the cell cycle, favouring uncontrolled growth of the tumour cells. This is clearly a mutation that is both antigenic and oncogenic. In addition to SK29-MEL, 1 of 28 melanomas that were tested carried this mutation.

CONCLUSIONS

The identification of tumour rejection antigens and the genes encoding the antigenic peptides opens up new possibilities for the active immunisation of cancer patients. The patients liable to benefit from immunisation with a defined antigen can be identified by HLA typing and by assessing the expression of antigen-encoding genes on a tumour sample.

Differentiation antigens are a major cause of concern. The presence of CTL against these antigens reflects a breach in tolerance towards self products. Vitiligo is sometimes associated with melanoma and reported to be of good prognostic value.[46] T cells directed against differentiation antigens may have contributed to the destruction of melanocytes. Post-vaccine immunity might be much stronger than spontaneous T cell responses, and may lead to important adverse effects.

Immunisation of patients against one of the antigens encoded by the *MAGE, BAGE* or *GAGE* genes should not cause autoimmune side effects caused by the expression of the relevant gene in the testis. Male germline cells, where expression of these genes appears to be restricted, do not express class I molecules.[47] Therefore, gene expression should not result in antigen presentation. These conclusions were further strengthened by immunisation studies of male mice against the tumour antigen encoded by mouse gene *P1A*, which is also expressed in testis. Immunised male mice showed a strong CTL response and did not show any testicular inflammation (C. Uyttenhove, unpublished results).

Antigens generated by point mutations are expected to be unique for an individual tumour or restricted to a very few. This should make it difficult to develop cancer therapeutic vaccines based on these antigens.

Clinical trials based on some of the tumour antigens that we have mentioned are in progress.[48] Little is known about efficient modes of immunisation, but it is our hope that responses will be obtained in some patients.

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Key words: Melanoma, Tumour antigen, MAGE, Immunotherapy.

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