

Human Tumor Antigens Recognized by T Lymphocytes

By Thierry Boon and Pierre van der Bruggen

From the Ludwig Institute for Cancer Research, Brussels Branch, B-1200 Brussels, Belgium; and Cellular Genetics Unit, Université Catholique de Louvain, B-1200 Brussels, Belgium

We have come a long way since the identification of the first human tumor antigen recognized by autologous CTL. This report provides a brief appraisal of these antigens and their potential for cancer immunotherapy. Our comments will be restricted to nonviral antigens.

The initial work carried out on mouse tumors revealed two possible mechanisms for generating new antigens that might be sufficiently tumor-specific to be of relevance to immunotherapy. The first mechanism involved a point mutation, and the second involved the transcriptional activation of a gene not expressed in normal tissues (1–3). Subsequent work on mouse tumors provided two interesting examples of tumor antigens resulting from point mutations (4, 5).

Tumor-specific Shared Antigens. Three families of genes that appear to code for highly specific tumor antigens have been identified so far, namely, the *MAGE*, *BAGE*, and *GAGE* genes (6–9). These genes are frequently expressed in a wide range of tumor types such as melanoma, lung carcinoma, sarcoma, and bladder carcinoma, but very rarely in other tumor types such as brain tumors, renal carcinoma, and leukemia (7, 10–12). The only normal tissues where expression of these genes has been observed are testis and placenta (7). Starting from CTL clones obtained by stimulating lymphocytes with an autologous melanoma cell line, six antigens encoded by *MAGE-1*, *MAGE-3*, *BAGE*, and *GAGE* have been identified (8, 9, 13–15). For these six antigens, both the presenting HLA molecule and the antigenic peptide have been completely defined. Remarkably, all the relevant CTL were derived from the same melanoma patient, a patient with metastatic disease who enjoyed an extraordinarily favorable clinical course. Blood samples from several patients with a tumor expressing some or all of these genes were tested, and no such CTL were obtained by stimulating the lymphocytes with autologous tumor cells.

More than 60% of Caucasian melanoma patients bear one of the presently defined antigens encoded by *MAGE*, *BAGE*, and *GAGE*. For other cancers such as head and neck tumors and bladder cancer, the frequencies range from 40% to 28%. For several reasons, it appears increasingly unlikely that immunization of patients against one of these antigens will cause harmful immunological side effects caused by the expression of the relevant gene in the testis. First, this expression appears to occur in germline cells, more precisely spermatocytes and spermatogonia (16). A similar observation has been made with the mouse equiv-

alent of a *MAGE* gene by in situ hybridization (17). Because these germline cells do not express classical MHC class I molecules, gene expression should not result in antigen expression (18). These conclusions are further strengthened by immunization studies carried out with mouse tumor antigen P815A, which is encoded by a gene that is also expressed only in the testis. After immunization with P815 tumor cells, which carry this antigen, male mice produced a strong CTL response. No inflammation of the testis was observed in the following months, and the fertility of these mice was normal (Uyttenhove, C., manuscript in preparation).

A new mode of origin for antigens that are also tumor-specific shared antigens is described in this issue (19). Here, it seems that a gene that is ubiquitously expressed, namely, *N*-acetyl-glucosaminyltransferase V, contains an intron that appears to carry near its end a promoter that is activated only in melanoma cells. This atypical activation occurs in >50% of melanomas. This produces a message containing a new open reading frame, which codes for the antigenic peptide in its intronic part.

Some CTL directed against breast, ovarian, and pancreatic carcinomas recognize an epitope of mucin, a surface protein composed of multiple tandem repeats of 20 amino acids (20–23). Whereas in normal cells mucin is heavily glycosylated, in these tumors the peptide repeats are unmasked by underglycosylation, resulting in CTL recognition. Remarkably, this recognition, which depends on the presence of multiple repeats, occurs in the absence of HLA restriction. The presence of this epitope was recently reported on myeloma cells, and mucin-specific CTL were isolated from the blood of a myeloma patient (24). These mucin antigens appear to be very specific for tumor cells, and the lack of HLA restriction should facilitate therapeutic vaccination trials.

Differentiation Antigens. The observation that autologous CTL can be generated readily against differentiation antigens present on normal melanocytes as well as melanoma cells was unexpected. Four genes encoding melanoma differentiation antigens have been identified: tyrosinase, Melan-A/Mart-1, gp100, and gp75 (25–30). Most of the identified antigenic peptides are presented by HLA-A2, but other HLA-peptide combinations have been found (29–38). One tyrosinase peptide is presented by HLA-DR4 to CD4 T cells (34).

The pattern of CTL precursors directed against these dif-

ferentiation antigens appears to be very different from that observed with the MAGE-like antigens. Here, most melanoma patients have CTL precursors that can be readily restimulated in vitro with autologous tumor cells (33, 39). TIL populations also contain these CTL (32). How these findings affect the immunotherapy potential of these antigens is unclear. The fact that many patients carry CTL precursors against these antigens implies that active immunization resulting in an increase in the number of these CTL should be possible. On the other hand, the fact that many of these patients have progressive disease suggests that these CTL are not very effective.

There is concern for the potential side effects of active or passive immunization against melanoma differentiation antigens. Not so much for the skin, where vitiligo caused by the destruction of melanocytes might occur, but for the uvea where melanocytes are present in the choroid layer. Vitiligo, however, has been associated with good prognoses in melanoma and also with adoptive transfer of TILs, without noticeable eye lesions (35, 37, 40, 41). Carefully devised immunotherapy trials based on these antigens therefore seem permissible.

Antigens Specific for Individual Tumors. Point mutations also generate antigens recognized on melanoma by autologous CTL. As was seen with the mouse antigens induced by mutagens, the mutations are located in the region coding for the antigenic peptide, enabling it to bind to the MHC molecule or generating a new epitope. A very interesting example is the point mutation of cyclin-dependent kinase 4 (42), which prevents this protein from binding to p16, thereby increasing the probability of its binding to the cyclin molecule and phosphorylating Rb, so that the E2F transcription factor is released and activates genes required for entry into the S phase of the cell cycle. This is clearly a mutation that is both antigenic and oncogenic. In addition to the melanoma tumor where the antigen was first identified, 1 out of 28 melanomas that were tested carried this mutation, confirming the oncogenic potential of this mutation. The amino acid change generated by this mutation enables the peptide to bind to the HLA-A2 presenting molecule. Another interesting point mutation produces a new antigenic peptide which, remarkably, is partially encoded by the 5' end of an intron (43). In this instance, the mutation generates a new epitope. Finally, this issue contains a report describing an antigenic peptide produced by a mutation in the β -catenin gene, which codes for a cell surface adhesion molecule. This mutation creates an anchor residue enabling the peptide to bind to HLA-A24 (44).

The antigens generated by point mutations ought to be absolutely specific for the tumor cells, and the CTL precursors directed against these antigens should not have undergone any of the depletion or anergy that accompany natural tolerance. On the other hand, they are expected to be unique for an individual tumor or restricted to very few. This should make it difficult to develop cancer therapeutic vaccines based on these antigens. But one should not exclude the possibility that technological progress may one

day make the identification of such antigens so easy that strictly individual immunogens will become a realistic possibility.

Ubiquitous Antigens. Some antigens that are recognized by autologous CTL stimulated in vitro with tumor cells appear to be encoded by genes that are ubiquitously expressed (45). We have identified more than six such genes, some of which show a significant degree of overexpression in the tumor cells (Brichard, V., P.G. Coulie, and P. van der Bruggen, personal communications). Unless these genes show a much higher degree of expression in tumor cells, as is observed with the *HER-2/neu* gene (46–49), one does not see how these antigens could be used for immunotherapy.

“Reverse Immunology.” Two antigens encoded by *MAGE* genes were identified by “reverse immunology:” starting from the sequence of the putative protein, candidate peptide sequences carrying consensus anchor motives for a certain HLA were located (50). These peptides were synthesized and some of them were found to effectively bind to the HLA molecule. Peptide-pulsed cells were then used to stimulate lymphocytes obtained from normal individuals carrying the relevant HLA-type, and CTL were obtained that lysed not only peptide-pulsed cells but also cells expressing the appropriate *MAGE* gene (51–53). In several instances, however, we have obtained CTL that recognize peptide-pulsed cells, but not the cells that express the relevant genes.

On the basis of the observation that a point mutation can generate new antigens (1, 2), this approach has also been used to identify antigens encoded by mutated oncogenes. CD8⁺ clones from a colon carcinoma patient, stimulated with a 25-amino acid mutated ras peptide, were capable of lysing a colon carcinoma line expressing a ras gene carrying this mutation (54). Mouse CD8⁺ CTL, obtained after in vivo immunization with a mutated p53 peptide, were likewise capable of lysing H-2-matched cells expressing the mutated p53 gene (55). Here again, after stimulation with a mutated peptide, several groups have obtained CTL that recognize only peptide-pulsed cells (56–59).

Will the antigens identified by direct immunology (i.e., by CTL resulting from tumor cell stimulation of autologous lymphocytes) prove to be superior for immunotherapy to those identified by reverse immunology involving stimulation with peptide-pulsed cells? We do not believe that this possibility can be ignored, since the lymphocytes that were stimulated with autologous tumor cells may have preferentially responded to the first group of antigens, because these antigens are better able to induce a CTL response, either because they are more abundant or because CTL with receptors of high affinity exist against them. But only clinical trials will provide definitive evidence regarding the usefulness of the two types of antigens.

Some of the tumor antigens that we have mentioned are in early stages of clinical study. There is little doubt that the coming years will witness a large number of clinical trials involving peptides, proteins, and recombinant defective vi-

ruses. It is our hope that responses will be obtained in some patients (60), and that the careful study of the lymphocytes and the tumor cells of these patients will produce a rich

harvest of additional antigens and a better understanding of what constitutes an effective antitumor response.

Address correspondence to Thierry Boon, Ludwig Institute for Cancer Research, Brussels Branch, 74 Avenue Hippocrate-UCL 74.59, B-1200 Brussels, Belgium.

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