TUMOUR ANTIGENS

A new look for the 1990s

Drew M. Pardoll

THIS is emerging as a banner year for tumour antigens, the latest evidence coming in the form of papers by Mandelboim *et al.*[1] and Cox *et al.*[2]. These two groups report the first successful identification, using strictly biochemical approaches, of tumour peptide antigens restricted by class I molecules of the major histocompatibility complex (MHC). Not only does this and related work bear on our understanding of how the immune system interacts with tumour cells, it also has implications for the development of antigen-specific immunotherapies.

Tumour antigens have a new look in the 1990s, one which stems from a change in ways of fishing for them. In the 1980s antibodies were the hook, but these days immunologists are hunting with T cells. T cells are the critical mediators of tumour specificity in developing adoptive immunotherapy and cancer vaccines, which makes sense in the light of how peptides derived from proteins in any cellular compartment can associate with MHC class I molecules to be presented to $CD8^+$ (cytotoxic) T cells. Consequently, the universe of potential tumour antigens recognizable by T cells is vast.

Without any prior clues, the task of identifying these antigens is technically daunting. The T-cell receptor (TCR) cannot be used as a reagent for affinity purification, because only a tiny fraction of MHC molecules will be occupied by the cognate peptide, and because the affinity of the TCR for its peptide–MHC complex is low. To circumvent this roadblock, Boon and colleagues[3,4] drew a page from bacterial genetics in developing the first standardized strategy for cloning genes encoding tumour antigens recognized by $CD8^+$ T cells. Once the target gene has been identified, the region encoding the epitope can be narrowed down and ultimately candidate peptides are synthesized to determine which most effectively stimulates the tumour-specific T cell. This approach has been used[5–11] to net a fistful of new human melanoma antigens (of which more below).

The biochemical strategy adopted by Mandelboim *et al.*[1] and Cox *et al.*[2] begins with the standard procedure of acid-eluting peptides bound to MHC class I from tumour cells, followed by fractionation with reversed-phase high-performance liquid chromatography (HPLC)[12,13]. Bioactive peptides recognized by the tumour-specific CTL are identified by adding the HPLC fractions to an antigen-processing mutant predominantly expressing empty class I molecules on the surface. Free peptides bind to the empty molecules so that fractions that sensitize the surrogate target for lysis by the tumour-specific T cells can be identified, further purified and ultimately sequenced. This approach is not for the faint-hearted — the huge number of different MHC class I-associated peptides (some 10,000–50,000 per cell) makes the task of identifying the single cognate peptide a biochemist's version of finding a needle in a haystack.

MHC class I-restricted tumour antigens

Tumour	Antigen	Normal adult tissue distribution	Genetically altered in tumour?	Source of T cells
Murine P815 mastocytoma	P1A	Testes	No	Vaccinated mice
Murine Lewis lung carcinoma	Connexin 37	Lungs	Yes*	Vaccinated mice
Human melanoma	MAGE-1	Testes	No	Peripheral blood lymphocytes from vaccinated patient
Human melanoma	MAGE-3	Testes	No	Peripheral blood lymphocytes from vaccinated patient
Human melanoma	MART 1/Aa	Melanocytes	No	Peripheral blood lymphocytes, tumour-infiltrating lymphocytes
Human melanoma	gp100	Melanocytes	No	Tumour-infiltrating lymphocytes, draining lymph node
Human melanoma	Tyrosinase	Melanocytes	No	Peripheral blood lymphocytes, tumour-infiltrating lymphocytes

*Unusual genetic alteration consisting of three consecutive nucleotide substitutions.

Mandelboim *et al.*[1] had the simpler task, probably because their tumour antigen represented an exceptionally large needle. They used bulk CTL cultures to identify a tumour antigen from the spontaneously arising murine Lewis lung carcinoma. This line was produced by immunizing mice with a whole-cell vaccine genetically engineered to express increased levels of an autologous MHC class I molecule. Edman sequencing of a single bioactive fraction turned up a predominant octapeptide sequence which matched that of the gap junction protein, connexin 37, in 7 of 8 positions. In this case, the most highly represented peptide indeed turned out to be the immunologically active peptide. This result indicates that the altered connexin 37 peptide constituted at least 50 per cent of the total material in the bioactive fraction, and is thus quite dominant among the total MHC class I-associated peptides on Lewis lung carcinoma cells.

A more complex picture emerges from the paper of Cox *et al.*[2], who used peptide fractionation to identify a shared, HLA-A2-restricted melanoma peptide recognized by CTL lines derived from T cells from patients' lymph nodes. Because each of several bioactive fractions contained over 50 peptides on mass spectroscopic (MS) analysis, a second-dimension HPLC fractionation, using different elution conditions, was required. At this point, tandem MS was employed to separate and then sequence a number of peptides in one of the bioactive fractions. The first MS was used to select and transfer individual peptides to the second, in which they were sequenced by a fragmentation technique. The third peptide sequenced, derived from the melanocyte-specific membrane protein, gp100, reconstituted recognition by melanoma-specific T-cell lines from four separate patients, thereby implicating it as a common, shared melanoma antigen.

At several hundred copies per cell, or roughly 0.1 per cent of total MHC-bound peptide, gp100 is apparently present at a much lower level than the Lewis lung peptide. Interestingly, despite its potency in stimulating T cells, it was estimated to bind relatively poorly to HLA-A2, implying that the affinity of the peptide–A2 complex for TCR is quite high. This result suggests that the peptide is efficiently loaded onto the MHC molecule via the endogenous pathway, despite a relatively high off-rate once bound. Consistent with a report that affinity of the peptide–MHC complex for TCR is an important determinant of T cell reactivity[14], these findings raise a cautionary flag about simply measuring MHC binding to identify the best epitopes for vaccine generation. ▶

Given the lack of cross-reactivity among tumour-specific T-cell lines generated against murine skin tumours induced by ultraviolet light[15], one might have predicted that most melanoma-reactive T cells would recognize bona fide tumour-specific antigens derived from mutated genes. Indeed, expectations that tumour-specific genetic alterations could produce tumour-specific peptides have launched a number of fishing expeditions for T cells recognizing peptide products of mutated oncogenes or tumour-suppressor genes. Nonetheless, the limitations on peptide binding to MHC class I molecules would restrict the general usefulness of peptide vaccines derived from an oncogene or tumour-suppressor gene.

Known tumour antigens are listed in the table; notably, all were identified without biasing the search towards a specific gene product. As the targets of T cells derived from either vaccinated or tumour-bearing individuals, they probably represent antigens against which tolerance is least stringently maintained. This notion is strengthened by the fact that many of the melanoma antigens were independently identified from different patients using different sources of T cells for the screening.

Some interesting themes emerge from the table. First, none of the antigens arise from the products of known oncogenes or tumour-suppressor genes. The only one stemming from an apparently mutated gene is the connexin 37 peptide in Lewis lung carcinoma. However, the reported Cys→Gln conversion can only be generated by nucleotide substitutions at all three codon positions. Such an alteration is highly unusual, and may instead represent a polymorphism in the mouse in which the tumour originally arose, or an event occurring sometime during the decades of passage *in vitro*.

Remarkably, none of the remainder are tumour-specific neoantigens. Instead, they fall into two categories. Antigens of the P1A and MAGE family are not expressed in any normal adult tissues (with the exception of testes), and are possibly developmental antigens re-expressed during the process of tumorigenesis. Indeed, MAGE-1 can be activated by demethylating agents such as 5-azacytidine; the altered methylation state commonly observed in cancers may thus account for their activation. Its relative tumour specificity makes MAGE-1 an excellent potential vaccine target. Subsequent searches have failed to reveal many patients with MAGE-1 reactive T cells, indicating that it represents a nondominant tumour antigen. The remaining melanoma antigens (tyrosinase, gp100, MART 1/Aa) are differentiation antigens specific to the melanocyte lineage. gp100 and MART 1/Aa seem to be dominant; a single peptide in each is recognized by T cells from many HLA-A2$^+$ patients.

Why don't the T cells from these immunized animals or patients recognize tumour-specific neoantigens? The reason may be that when a neoantigen arises within a tumour, it is tolerated by the immune system equivalently to a tissue-specific antigen. So the efficiency with which a particular epitope is processed, presented and ultimately recognized by a TCR is a much more critical determinant of immunological reactivity than is neoantigenicity. As ubiquitous as genetically altered proteins appear to be in cancer cells, they are still a relatively minor source of total MHC class I-associated peptides. Only if a tumour-specific neoepitope happens to be effectively processed and presented will it qualify as a dominant target for T-cell immunity. The altered connexin 37 peptide in Lewis lung carcinoma is probably one such example, as shown by its unusually high representation among the peptides bound to MHC class I.

Another determinant of immunogenicity is the tissue type in which the potential antigen is expressed. Experiments with transgenic mice suggest that mechanisms of tolerance to the same antigen can differ depending on which tissue type it is expressed in[16]. It is no coincidence that most known T-cell tumour antigens are derived from human melanoma. This tumour appears to be particularly immunogenic; among human tumours, it is the most sensitive to immunotherapy and is the easiest to generate T cells against. The rules of antigenicity may be quite different for tumours of different histological origin.

The main clinical value of T-cell-defined tumour antigens is in vaccine development. If we can identify targets against which tolerance can most readily be broken, these would probably be the best candidates for generating successful therapies. At first glance, vaccines using antigens that are not truly tumour specific would seem to be inappropriate because of their potential for generating autoimmunity. Often, however, the tissues from which common tumours arise are themselves dispensable. The prostate is probably the best example. In the case of melanoma, some melanocyte-specific antigens are also expressed in certain cells of the retina, inner ear and brain; whereas melanoma patients receiving immunotherapy occasionally develop vitiligo, they do not develop abnormalities of the visual, vestibular, or central nervous system. Also, the normal tissue counterparts of many tumours exhibit extremely low levels of MHC class I, thereby cloaking them from recognition by tissue-specific CTL. So a wide enough window for therapy may occur, even if tolerance is broken against tissue-specific antigens.

As other antigen targets of tumour-reactive T cells are identified, approaches to generate effective immune responses against them must be evaluated. There has been much interest in peptide vaccines because of their ability to generate CTL responses, though established tumours have yet to be cured with a single peptide vaccine. Also, individual peptides will only have general therapeutic value if they can be efficiently presented by common MHC alleles such as HLA-A2. The finding that some of the melanoma antigens produce several peptide targets in patients with different HLA haplotypes provides a basis for vaccine strategies that use the entire antigen and allow the individual's own MHC alleles to choose the epitope.

Finally, the emphasis on MHC class I-restricted tumour antigens has overshadowed the equally important MHC class II-restricted CD4 response. Adoptive transfer experiments, as well as analyses of genetically modified tumour vaccines, show that CD4$^+$ cells are every bit as necessary for generating efficient systemic antitumour responses as are CD8$^+$ cells[17,18]. Recently, a shared MHC class II-restricted melanoma antigen has been identified as tyrosinase[19]. The notion that the same antigen encodes both MHC class I and class II epitopes makes sense, given that both class I and class II tumour antigens appear to be presented by antigen-presenting cells derived from bone marrow during induction of the immune response[20]. Linkage between class I and class II restricted epitopes would therefore produce the most efficient interaction between CD4$^+$ and CD8$^+$ T cells, and it would seem that the most effective cancer vaccines will use natural or chimaeric antigens containing both MHC class I and class II epitopes. □

Drew M. Pardoll is in the Oncology Center, John Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

1. Mandelboim, O. *et al. Nature* **369**, 67–71 (1994).
2. Cox, A. L. *et al. Science* **264**, 716–719 (1994).
3. De Plaen, E. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 2274–2281 (1988).
4. Van den Eynde, B., Lethe, B., Van Pel, A., De Plaen, E. & Boon, T. *J. exp. Med.* **173**, 1373–1384 (1991).
5. Van der Bruggen, P. C. *et al. Science* **254**, 1643–1650 (1991).
6. Gaughler, B. *et al. J. exp. Med.* **179**, 921–930 (1994).
7. Brichard, V. *et al. J. exp. Med.* **178**, 489–495 (1993).
8. Coulie, P. *et al. J. exp. Med.* (in the press).
9. Bakker, A. B. H. *et al. J. exp. Med.* **179**, 1005–1009 (1994).
10. Kawakami, Y. *et al. Proc. natn. Acad. Sci. U.S.A.* **91**, 3515–3519 (1994).
11. Kawakami, Y. *et al. J. exp. Med.* (in the press).
12. Rotzscheke, O. *et al. Nature* **348**, 252–254 (1990).
13. Van Bleek, G. & Nathenson, S. *Nature* **348**, 213–215 (1990).
14. Sykulev, Y., Brunmark, A., Jackson, M., Cohen, R. J. & Peterson, P. A. *Immunity* **1**, 15–22 (1994).
15. Ward, P., Koeppen, H., Hurteau, T. & Schreiber, H. *J. exp. Med.* **170**, 217–232 (1989).
16. Ferber, I. *et al. Science* **263**, 674–676 (1994).
17. Greenberg, P. D., Kern, D. E. & Cheever, M. A. *J. exp. Med.* **161**, 1122–1134 (1985).
18. Dranoff, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **90**, 3539–3543 (1993).
19. Topalian, S. L. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
20. Huang, A. Y. C. *et al. Science* **264**, 961–964 (1994).