Commentary

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 equivalent 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-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/96/03/725/05 $2.00
Volume 183 March 1996 725–729

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.

Received for publication 23 January 1996.

References

1. De Plaen, E., C. Lurquin, A. Van Pel, B. Mariamé, J.-P. Szikora, T. Wölfel, C. Sibille, P. Chomez, and T. Boon. 1988. Immunogenic (tum$^-$) variants of mouse tumor P815: cloning of the gene of tum$^-$ antigen P91A and identification of the tum$^-$ mutation. *Proc. Natl. Acad. Sci. USA.* 85:2274–2278.
2. Lurquin, C., A. Van Pel, B. Mariamé, E. De Plaen, J.-P. Szikora, C. Janssens, M. Reddehase, J. Lejeune, and T. Boon. 1989. Structure of the gene coding for tum$^-$ transplantation antigen P91A. A peptide encoded by the mutated exon is recognized with Ld by cytolytic T cells. *Cell.* 58:293–303.
3. Van den Eynde, B., B. Lethé, A. Van Pel, E. De Plaen, and T. Boon. 1991. The gene coding for a major tumor rejection antigen of tumor P815 is identical to the normal gene of syngeneic DBA/2 mice. *J. Exp. Med.* 173:1373–1384.
4. Mandelboim, O., G. Berke, M. Fridkin, M. Feldman, M. Eisenstein, and L. Eisenbach. 1994. CTL induction by a tumour-associated antigen octapeptide derived from a murine lung carcinoma. *Nature (Lond.).* 369:67–71.
5. Monach, P.A., S.C. Meredith, C.T. Siegel, and H. Schreiber. 1995. A unique tumor antigen produced by a single amino acid substitution. *Immunity.* 2:45–59.
6. van der Bruggen, P., C. Traversari, P. Chomez, C. Lurquin, E. De Plaen, B. Van den Eynde, A. Knuth, and T. Boon. 1991. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science (Wash. DC).* 254:1643–1647.
7. De Plaen, E., K. Arden, C. Traversari, J.J. Gaforio, J.-P. Szikora, C. De Smet, F. Brasseur, P. van der Bruggen, B. Lethé, C. Lurquin, et al. 1994. Structure, chromosomal localization and expression of twelve genes of the MAGE family. *Immunogenetics.* 40:360–369.
8. Boël, P., C. Wildmann, M.-L. Sensi, R. Brasseur, J.-C. Renauld, P. Coulie, T. Boon, and P. van der Bruggen. 1995. BAGE, a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity.* 2: 167–175.
9. Van den Eynde, B., O. Peeters, O. De Backer, B. Gaugler, S. Lucas, and T. Boon. 1995. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J. Exp. Med.* 182:689–698.
10. Brasseur, F., M. Marchand, R. Vanwijck, M. Hérin, B. Lethé, P. Chomez, and T. Boon. 1992. Human gene MAGE-1, which codes for a tumor rejection antigen, is expressed by some breast tumors. *Int. J. Cancer.* 52:839–841.
11. Brasseur, F., D. Rimoldi, D. Liénard, B. Lethé, S. Carrel, F. Arienti, L. Suter, R. Vanwijck, A. Bourlond, Y. Humblet, A. Vacca, et al. 1995. Expression of MAGE genes in primary and metastatic cutaneous melanoma. *Int. J. Cancer.* 63:375–380.
12. Weynants, P., B. Lethé, F. Brasseur, M. Marchand, and T. Boon. 1994. Expression of MAGE genes by non-small-cell lung carcinomas. *Int. J. Cancer.* 56:826–829.
13. Traversari, C., P. van der Bruggen, I.F. Luescher, C. Lurquin, P. Chomez, A. Van Pel, E. De Plaen, A. Amar-Costesec, and T. Boon. 1992. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. *J. Exp. Med.* 176:1453–1457.
14. van der Bruggen, P., J.-P. Szikora, P. Boël, C. Wildmann, M. Somville, M. Sensi, and T. Boon. 1994. Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing HLA-Cw*1601. *Eur. J. Immunol.* 24: 2134–2140.
15. Gaugler, B., B. Van den Eynde, P. van der Bruggen, P. Romero, J.J. Gaforio, E. De Plaen, B. Lethé, F. Brasseur, and T. Boon. 1994. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. *J. Exp. Med.* 179:921–930.
16. Takahashi, K., S. Shichijo, M. Noguchi, M. Hirohata, and K. Itoh. 1995. Identification of MAGE-1 and MAGE-4 proteins in spermatogonia and primary spermatocytes of testis. *Cancer Res.* 55:3478–3482.
17. Chomez, P., R. Williams, O. De Backer, T. Boon, and B. Vennström. 1995. The Smage gene family is expressed in post-meiotic spermatids during mouse germ cell differentiation. *Immunogenetics.* 43:97–100.
18. Haas, G.G., Jr., O.J. D'Cruz, and L.E. De Bault. 1988. Distribution of human leukocyte antigen-ABC and -D/DR antigens in the unfixed human testis. *Am. J. Reprod. Immunol. Microbiol.* 18:47–51.
19. Guilloux, Y., S. Lucas, V.G. Brichard, A. Van Pel, C. Viret, E. De Plaen, F. Brasseur, B. Lethé, F. Jotereau, and T. Boon. 1996. A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the *N*-acetylglucosaminyltransferase V gene. *J. Exp. Med.* 183:1173–1183.
20. Gendler, S., J. Taylor-Papadimitriou, T. Duhig, J. Rothbard, and J. Burchell. 1988. A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeats. *J. Biol. Chem.* 263:12820–12823.
21. Barnd, D.L., L.A. Kerr, R.S. Metzgar, and O.J. Finn. 1988. Human tumor-specific cytotoxic T cell lines generated from tumor-draining lymph node infiltrate. *Transplant. Proc.* 20: 339–341.
22. Lancaster, C.A., N. Peat, T. Duhig, D. Wilson, J. Taylor-

Papadimitriou, and S.J. Gendler. 1990. Structure and expression of the human polymorhic epithelial mucin gene: an expressed VNTR unit. *Biochem. Biophys. Res. Commun.* 173: 1019–1029.

23. Jerome, K.R., N. Domenech, and O.J. Finn. 1993. Tumor-specific cytotoxic T cell clones from patients with breast and pancreatic adenocarcinoma recognize EBV-immortalized B cells transfected with polymorphic epithelial mucin cDNA. *J. Immunol.* 151:1654–1662.
24. Takahashi, T., Y. Makiguchi, Y. Hinoda, H. Kakiuchi, N. Nakagawa, K. Imai, and A. Yachi. 1994. Expression of MUC1 on myeloma cells and induction of HLA-unrestricted CTL against MUC1 from a multiple myeloma patient. *J. Immunol.* 153:2102–2109.
25. Brichard, V., A. Van Pel, T. Wölfel, C. Wölfel, E. De Plaen, B. Lethé, P. Coulie, and T. Boon. 1993. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.* 178:489–495.
26. Coulie, P.G., V. Brichard, A. Van Pel, T. Wölfel, J. Schneider, C. Traversari, S. Mattei, E. De Plaen, C. Lurquin, J.-P. Szikora, et al., 1994. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.* 180:35–42.
27. Kawakami, Y., S. Eliyahu, C.H. Delgado, P.F. Robbins, L. Rivoltini, S.L. Topalian, T. Miki, and S.A. Rosenberg. 1994. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc. Natl. Acad. Sci. USA.* 91:3515–3519.
28. Bakker, A.B.H., M.W.J. Schreurs, A.J. de Boer, Y. Kawakami, S.A. Rosenberg, G.J. Adema, and C.G. Figdor. 1994. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J. Exp. Med.* 179:1005–1009.
29. Cox, A.L., J. Skipper, Y. Chen, R.A. Henderson, T.L. Darrow, J. Shabanowitz, V.H. Engelhard, D.F. Hunt, and C.L. Slingluff, Jr. 1994. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science (Wash. DC).* 264:716–719.
30. Wang, R.-F., P.F. Robbins, Y. Kawakami, X.-Q. Kang, and S.A. Rosenberg. 1995. Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31–restricted tumor-infiltrating lymphocytes. *J. Exp. Med.* 181:799–804.
31. Wölfel, T., A. Van Pel, V. Brichard, J. Schneider, B. Seliger, K.-H. Meyer zum Büschenfelde, and T. Boon. 1994. Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. *Eur. J. Immunol.* 24: 759–764.
32. Robbins, P.F., M. El-Gamil, Y. Kawakami, and S.A. Rosenberg. 1994. Recognition of tyrosinase by tumor-infiltrating lymphocytes from a patient responding to immunotherapy. *Cancer Res.* 54:3124–3126.
33. Brichard, V.G., J. Herman, A. Van Pel, C. Wildmann, B. Gaugler, T. Wölfel, T. Boon, and B. Lethé. 1996. A tyrosinase nonapeptide presented by HLA-B44 is recognized on a human melanoma by autologous cytolytic T lymphocytes. *Eur. J. Immunol.* 26:224–230.
34. Topalian, S.L., L. Rivoltini, M. Mancini, N.R. Markus, P.F. Robbins, Y. Kawakami, and S.A. Rosenberg. 1994. Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. *Proc. Natl. Acad. Sci. USA.* 91:9461–9465.
35. Kawakami, Y., S. Eliyahu, C.H. Delgado, P.F. Robbins, K. Sakaguchi, E. Appella, J.R. Yannelli, G.J. Adema, T. Miki, and S.A. Rosenberg. 1994. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. *Proc. Natl. Acad. Sci. USA.* 91:6458–6462.
36. Kawakami, Y., S. Eliyahu, K. Sakaguchi, P.F. Robbins, L. Rivoltini, J.R. Yannelli, E. Appella, and S.A. Rosenberg. 1994. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2–restricted tumor infiltrating lymphocytes. *J. Exp. Med.* 180:347–352.
37. Kawakami, Y., S. Eliyahu, C. Jennings, K. Sakaguchi, X. Kang, S. Southwood, P.F. Robbins, A. Sette, E. Appella, and S.A. Rosenberg. 1995. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. *J. Immunol.* 154:3961–3968.
38. Castelli, C., W.J. Storkus, M.J. Maeurer, D.M. Martin, E.C. Huang, B.N. Pramanik, T.L. Nagabhushan, G. Parmiani, and M.T. Lotze. 1995. Mass spectrometric identification of a naturally processed melanoma peptide recognized by $CD8^+$ cytotoxic T lymphocytes. *J. Exp. Med.* 181:363–368.
39. Sensi, M.L., C. Traversari, M. Radrizzani, S. Salvi, C. Maccalli, R. Mortarini, L. Rivoltini, C. Farina, G. Nicolini, T. Wölfel, et al. 1995. Cytotoxic T-lymphocyte clones from different patients display limited T-cell-receptor variable-region gene usage in HLA-A2-restricted recognition of the melanoma antigen Melan-A/MART-1. *Proc. Natl. Acad. Sci. USA.* 92:5674–5678.
40. Bystryn, J.-C., R. Darrell, R.J. Friedman, and A. Kopf. 1987. Prognostic significance of hypopigmentation in malignant melanoma. *Arch. Dermatol.* 123:1053–1055.
41. Richards, J.M., N. Mehta, K. Ramming, and P. Skosey. 1992. Sequential chemoimmunotherapy in the treatment of metastatic melanoma. *J. Clin. Oncol.* 10:1338–1343.
42. Wölfel, T., M. Hauer, J. Schneider, M. Serrano, C. Wölfel, E. Klehmann-Hieb, E. De Plaen, T. Hankeln, K.-H. Meyer zum Büschenfelde, and D. Beach. 1995. A $p16^{INK4a}$-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science (Wash. DC).* 269:1281–1284.
43. Coulie, P.G., F. Lehmann, B. Lethé, J. Herman, C. Lurquin, M. Andrawiss, and T. Boon. 1995. A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. *Proc. Natl. Acad. Sci. USA.* 92:7976–7980.
44. Robbins, P.F., M. El-Gamil, Y.F. Li, Y. Kawakami, D. Loftus, E. Appella, and S.A. Rosenberg. 1996. A mutated β-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J. Exp. Med.* 183:1185–1192.
45. Robbins, P.F., M. el-Gamil, Y.F. Li, S.L. Topalian, L. Rivoltini, K. Sakaguchi, E. Appella, Y. Kawakami, and S.A. Rosenberg. 1995. Cloning of a new gene encoding an antigen recognized by melanoma-specific HLA-A24-restricted tumor-infiltrating lymphocytes. *J. Immunol.* 154:5944–5950.
46. Ioannides, C.G., B. Fisk, D. Fan, W.E. Biddison, J.T. Wharton, and C.A. O'Brian. 1993. Cytotoxic T cells isolated from ovarian malignant ascites recognize a peptide derived from the HER-2/neu proto-oncogene. *Cell. Immunol.* 151:225–234.
47. Fisk, B., B. Chesak, M.S. Pollack, J.T. Wharton, and C.G. Ioannides. 1994. Oligopeptide induction of a cytotoxic T lymphocyte response to HER-2/neu proto-oncogene in vitro. *Cell. Immunol.* 157:415–427.

48. Yoshino, I., G.E. Peoples, P.S. Goedegebuure, R. Maziarz, and T.J. Eberlein. 1994. Association of HER2/neu expression with sensitivity to tumor-specific CTL in human ovarian cancer. *J. Immunol.* 152:2393–2400.
49. Peoples, G.E., P.S. Goedegebuure, R. Smith, D.C. Linehan, I. Yoshino, and T.J. Eberlein. 1995. Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide. *Proc. Natl. Acad. Sci. USA.* 92: 432–436.
50. Rammensee, H.-G., T. Friede, and S. Stevanovic. 1995. MHC ligands and peptide motifs: first listing. *Immunogenetics.* 41: 178–228.
51. Celis, E., V. Tsai, C. Crimi, R. DeMars, P.A. Wentworth, R.W. Chesnut, H.M. Grey, A. Sette, and H.M. Serra. 1994. Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes. *Proc. Natl. Acad. Sci. USA.* 91:2105–2109.
52. van der Bruggen, P., J. Bastin, T. Gajewski, P.G. Coulie, P. Boël, C. De Smet, C. Traversari, A. Townsend, and T. Boon. 1994. A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. *Eur. J. Immunol.* 24:3038–3043.
53. Herman, J., P. van der Bruggen, I. Luescher, S. Mandruzzato, P. Romero, J. Thonnard, K. Fleischhauer, T. Boon, and P.G. Coulie. 1996. A peptide encoded by human gene MAGE-3 and presented by HLA-B44 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. *Immunogenetics.* In press.
54. Fossum, B., A.C. Olsen, E. Thorsby, and G. Gaudernack. 1995. $CD8^+$ T cells from a patient with colon carcinoma, specific for a mutant p21-Ras-derived peptide ($GLY^{13} \rightarrow$ ASP), are cytotoxic towards a carcinoma cell line harbouring the same mutation. *Cancer Immunol. Immunother.* 40:165–172.
55. Noguchi, Y., Y.-T. Chen, and L.J. Old. 1994. A mouse mutant p53 product recognized by $CD4^+$ and $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA.* 91:3171–3175.
56. Disis, M.L., J.W. Smith, A.E. Murphy, W. Chen, and M.A. Cheever. 1994. In vitro generation of human cytolytic T-cells specific for peptides derived from the HER-2/neu protooncogene protein. *Cancer Res.* 54:1071–1076.
57. Gedde-Dahl III, T., A. Spurkland, B. Fossum, A. Wittinghofer, E. Thorsby, and G. Gaudernack. 1994. T cell epitopes encompassing the mutational hot spot position 61 of p21 ras. Promiscuity in ras peptide binding to HLA. *Eur. J. Immunol.* 24:410–414.
58. Nijman, H.W., S.H. Van der Burg, M.P.M. Vierboom, J.G.A. Houbiers, W.M. Kast, and C.J.M. Melief. 1994. p53, a potential target for tumor-directed T cells. *Immunol. Lett.* 40:171–178.
59. Van Elsas, A., H.W. Nijman, C.E. Van der Minne, J.S. Mourer, W.M. Kast, C.J.M. Melief, and P.I. Schrier. 1995. Induction and characterization of cytotoxic T-lymphocytes recognizing a mutated p21ras peptide presented by HLA-A*0201. *Int. J. Cancer.* 61:389–396.
60. Marchand, M., P. Weynants, E. Rankin, F. Arienti, F. Belli, G. Parmiani, N. Cascinelli, A. Bourlond, R. Vanwijck, Y. Humblet, et al. 1995. Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. *Int. J. Cancer.* 63:883–885.