Int J Clin Lab Res (1997) 27: 81–86

REVIEW

B. J. Van den Eynde · T. Boon

Tumor antigens recognized by T lymphocytes

Accepted: 8 January 1997

Abstract In the last five years, knowledge of human tumor antigens recognized by autologous cytolytic T lymphocytes (CTL) has increased considerably. So far, genetic and biochemical approaches have led to the molecular identification of three classes of antigens. Most of these antigens consist of peptides that are presented to T cells by HLA molecules. The first class comprises antigens encoded by genes such as MAGE, BAGE, and GAGE, which are expressed in various tumors of different histological origins, but not in normal tissues other than testis. The second class represents differentiation antigens encoded by genes that are only expressed in melanoma and normal melanocytes like tyrosinase, Melan-A/MART-1, gp100 and gp75. The third class includes antigens produced by unique point mutations in genes that are ubiquitously expressed. In most cases, the antigenic peptide is encoded by the mutated region of the gene. A number of these antigens provide promising targets for new protocols of specific cancer immunotherapy.

Key words Cancer · Cytolytic T lymphocytes · Antigen · Peptide · Melanoma · Renal cell carcinoma · Immunotherapy · Vaccine

Introduction

It is about 40 years since Prehn and Main [1] demonstrated in animal models that the immune system is capable of rejecting tumor cells. Any progress in tumor immunology critically depended on: (1) understanding the effectors involved and their mechanisms of action and (2) the identification of target antigens on the tumor cells. In the 1970s, the essential role of T cells, and more particularly of $CD8^+$ cytolytic T cells (CTL), in tumor rejection was discovered, and tumor-specific CTL were isolated in various tumor models [2, 3]. In the 1980s, CTL directed against human tumors were also isolated, essentially against melanomas [4]. The early 1990s witnessed the molecular identification of a number of tumor antigens recognized by such CTL. This allows the design of immune manipulations specifically targeted at defined tumor antigens. This review will focus on the different antigens identified to date on human tumors, and will briefly discuss these new prospects of specific cancer immunotherapy.

B. J. Van den Eynde (✉)
Ludwig Institute for Cancer Research, Brussels Branch,
74 avenue Hippocrate, UCL 74.59,
B-1200 Brussels, Belgium

T. Boon
Cellular Genetics Unit,
Université Catholique de Louvain,
B-1200 Brussels, Belgium

Identification of peptides presented to CTL

Antigens recognized by CTL consist of peptides derived from endogenous proteins and presented by MHC molecules. Three methods have been used to identify the peptides presented to tumor-specific T cells. The first and most successful is a genetic approach based on the transfection of recombinant DNA libraries into cells expressing the MHC-presenting molecule, in order to isolate the gene encoding the antigen [5]. Once the gene is isolated, the antigenic peptide is deduced from the sequence of the putative protein [6, 7]. The second method is a biochemical purification of the peptides acid-eluted from the MHC class I molecules of the tumor cells. This method has met with limited success, essentially because the amount of peptide recovered in positive fractions is often too small to allow direct and reliable sequencing [8]. The third method is the reverse of the first two. Rather than a tumor-specific CTL, the starting point is the sequence of a protein known to be overexpressed or mutated in tumor cells. Peptides selected for their good binding to a given MHC class I molecule are loaded on antigen-presenting cells to stimulate lymphocytes in vitro [9–11]. Peptide-specific CTL can readily be

obtained by this procedure, but a number do not recognize HLA-matched tumor cells expressing the protein endogenously, either because of their low affinity or because the peptide itself is not processed or presented as such by the cell. Therefore, recognition of cells expressing the relevant HLA and the gene encoding the peptide is required to demonstrate that the peptide is a genuine tumor antigen.

Classification of tumor antigens

On the basis of their pattern of expression, tumor antigens can be classified into three groups. Antigens of the first group are encoded by genes that are expressed in many tumor cells but are silent in normal adult tissues, except testis. The second group consists of melanocyte differentiation antigens. Antigens of a third group are unique to individual tumors and result from mutations occurring in genes that are expressed in most normal tissues.

Tumor-specific shared antigens

Three families of genes that appear to code for highly specific tumor antigens have been identified to date, namely the MAGE, BAGE, and GAGE genes [5, 12–14]. 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 (Table 1) [12, 15–17]. The only normal tissues where expression of these genes has been observed are testis and placenta [12]. Starting from CTL clones obtained by stimulating lymphocytes with an autologous melanoma cell line, seven antigens encoded by MAGE-1, MAGE-3, BAGE, and GAGE have been identified [6, 11, 13, 14, 18–20]. For these seven antigens, both the presenting HLA molecule and the antigenic peptide have been completely defined (Table 2).

More than 60% of Caucasian melanoma patients bear one of the defined antigens encoded by MAGE, BAGE, and GAGE. For other cancers such as lung cancer, head

Table 1 Expression of genes MAGE-1, -3, BAGE, GAGE, and RAGE in tumor samples[a]

Histological type	Percentage of tumors positive for				
	MAGE-1	MAGE-3	BAGE	GAGE-1, 2	RAGE-1
Melanomas					
primary lesions	16	36	8	13	2
metastases	48	76	26	28	5
Non-small-cell lung carcinomas	49	47	4	19	0
Head and neck tumors	28	49	8	19	2
Bladder carcinomas	22	36	15	12	5
Sarcomas	14	24	6	25	14
Mammary carcinomas	18	11	10	9	1
Prostatic carcinomas	15	15	0	10	0
Colorectal carcinomas	2	17	0	0	0
Renal carcinomas	0	0	0	0	2
Leukemias and lymphomas	0	0	0	1	0
Testicular seminomas	4/6	3/6	1/6	5/6	0/3

[a] Expression was measured by reverse transcriptase-polymerase chain reaction on total RNA using primers specific for each gene

Table 2 Tumor-specific antigens shared by different tumors

Gene	Normal expression	MHC	Peptide	Position	Reference
MAGE-1	Testis	HLA-A1	EADPTGHSY	161–169	[6]
		HLA-Cw16	SAYGEPRKL	230–238	[18]
MAGE-3	Testis	HLA-A1	EVDPIGHLY	168–176	[19]
		HLA-A2	FLWGPRALV	271–279	[11]
		HLA-B44	MEVDPIGHLY	167–176	[20]
BAGE	Testis	HLA-Cw16	AARAVFLAL	2–10	[13]
GAGE-1/2	Testis	HLA-Cw6	YRPRPRRY	9–16	[14]
RAGE-1	Retina	HLA-B7	SPSSNRIRNT	11–20	[27]
GnTV (atypical transcript)	None	HLA-A2	VLPDVFIRC	38–64	[26]
HPV16E7	None	HLA-A2	YMLDLQPETT	11–20	[28]
		HLA-A2	LLMGTLGIV	82–90	[28]
		HLA-A2	TLGIVCPI	86–93	[28]

and neck cancer, and bladder cancer, the frequencies range from 40% to 28%. It appears increasingly unlikely that immunization of patients against one of these antigens will cause harmful immunological side-effects due to the expression of the relevant gene in testis. This expression appears to occur in germ-line cells, more precisely spermatocytes and spermatogonia [21]. A similar observation has been made with the mouse equivalent of a MAGE gene by in situ hybridization [22]. Because these germ-line cells do not express MHC class I molecules, gene expression should not result in antigen expression [23]. 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 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 [24].

Genes MAGE, BAGE, and GAGE are methylated in normal cells. Their expression in tumor cells appears to result from a genome-wide demethylation process that occurs in many cancers and is correlated with tumor progression [25].

A new origin for antigens that are also tumor-specific shared antigens has been described recently [26]. A gene that is ubiquitously expressed, namely *N*-acetyl-glucosaminyltransferase V (GnTV), contains an intron which itself appears to carry near its end a promoter that is activated only in melanoma cells. This atypical activation occurs in more than 50% of melanomas. It produces a message containing a new open reading frame, which codes for the antigenic peptide in its intronic part. This peptide is presented by HLA-A2 to melanoma-specific CTL (Table 2).

Recently, a new antigen recognized by CTL on a kidney tumor was identified. This antigen was found to be encoded by a previously unknown gene that was called RAGE [27]. This gene is silent in normal tissues except retina, and is expressed in a small proportion of tumors, mainly in sarcomas, bladder tumors, and melanomas (Table 1). Since most retinal cells do not express MHC class I molecules, this antigen is probably tumor specific, although formal proof of this will require the identification of the retinal cell type that expresses RAGE. The antigenic peptide recognized by the CTL has been identified. It is presented by HLA-B7 (Table 2).

Antigens derived from oncogenic viruses constitute another category of potentially useful tumor antigens, and the best example is the oncoprotein E7 of human papilloma virus 16 (HPV16). HPV DNA is detected in about 90% of squamous cell carcinomas of the human cervix and is believed to play a causative role in cervical carcinogenesis. HPV oncoproteins E6 and E7 are constitutively expressed in cervical tumor cells, and their expression is required for maintenance of the transformed state. Peptides derived from the HPV16 E7 oncoprotein and selected for their high binding affinity to HLA-A2 were used to stimulate CD8+ lymphocytes from normal individuals [28]. CTL clones obtained against three of these peptides were found to recognize not only peptide-pulsed cells but also a cervical carcinoma cell line expressing HLA-A2 and HPV16 E7, demonstrating that these peptides indeed correspond to genuine cervical carcinoma antigens (Table 2).

Differentiation antigens

A large number of CTL directed against human melanoma were found to recognize not only a majority of HLA-A2$^+$ melanomas but also HLA-A2$^+$ normal melanocytes [29]. The notion that such CTL recognize melanocyte differentiation antigens was confirmed when the antigens were identified at the molecular level. Four genes encoding melanocyte differentiation antigens were identified: tyrosinase, Melan-A/Mart-1, gp100, and gp75 [8, 30–34]. Most of the identified peptides are presented by HLA-A2, but other HLA-peptide combinations have been found (Table 3)

Table 3 Melanocyte differentiation antigens recognized by T cells on melanoma

Gene/protein	MHC	Peptide	Position	Reference
Tyrosinase	HLA-A2	MLLAVLYCL	1–9	[7]
	HLA-A2	YMNGTMSQV	369–377	[7]
		YMDGTMSQV[a]		[43]
	HLA-A24	AFLPWHRLF	206–214	[42]
	HLA-B44	SEIWRDIDF	192–200	[36]
	HLA-DR4	QNILLSNAPLGPQFP	56–70	[44]
		DYSYLQDSDPDSFQD	448–462	[44]
Pme117^{gp100}	HLA-A2	KTWGQYWQV	154–162	[40]
	HLA-A2	ITDQVPGSV	209–217	[40]
	HLA-A2	YLEPGPVTA[a]	280–288	[8]
	HLA-A2	LLDGTATLRL	457–466	[32]
	HLA-A2	VLYRYGSFSV	476–485	[40]
Melan-A^{MART-1}	HLA-A2	(E)AAGIGILTV[b]	26(7)–35	[39]
	HLA-A2	ILTVILGVL[a]	32–40	[41]
gp75^{TRP1}	HLA-A31	MSLQRQFLR	–[c]	[45]

[a] Natural peptide identified by elution from HLA-A2 molecules
[b] Different cytotoxic T lymphocytes preferentially recognize either the nonamer or the decamer
[c] Peptide translated from an alternative open reading frame

[7, 8, 34–42]. Tyrosinase peptide 369–377, which is presented by HLA-A2, presents an interesting post-translational modification that was observed when the naturally occurring peptide eluted from an HLA-$A2^+$ melanoma was analyzed [43]. The asparagine in position 3 is transformed into an aspartic acid residue, presumably because the asparagine was glycosylated and subsequently deglycosylated by an enzyme which removed the amino group with the glycan. Two other tyrosinase peptides are presented by HLA-DR4 to non-cytolytic CD4 T cells [37, 44]. The gp75 peptide presented by HLA-A31 is peculiar in that it is translated from an open reading frame that is different from the one encoding the gp75 protein [45].

The observation that melanocyte differentiation antigens are targets for anti-melanoma CTL is in line with previous reports of the presence of antibodies against melanocytic proteins in the sera of melanoma patients [46]. The multiplicity of T cell epitopes derived from melanocytic proteins and the recognition of some of these epitopes by CTL of many melanoma patients highlight the immunogenicity of these antigens. The role of such CTL in melanoma rejection is not clear, but is supported by the reported association of vitiligo with prolonged survival and spontaneous regression of melanoma [47, 48]. Moreover, the adoptive transfer in a melanoma patient of tumor-infiltrating lymphocytes directed against $Pmel17^{gp100}$ led to objective regressions of melanoma metastases [38, 40]. Nevertheless, potential side-effects of active or passive immunization against melanoma differentiation antigens must be evaluated carefully, not so much for the skin, where vitiligo is expected, but for the retina where melanocytes are present in the choroid layer. However, since melanoma patients with vitiligo and prolonged survival did not show noticeable eye lesions [38, 40, 47, 48], carefully devised immunotherapy trials based on these antigens seem permissible.

Unique antigens

Several years ago, the study of immunogenic variants of mouse tumors obtained by mutagenesis showed that point mutations in genes expressed ubiquitously could create antigenic epitopes recognized by CTL [49]. Subsequent work on mouse tumors provided two interesting examples of tumor antigens resulting from point mutations [50, 51]. This mechanism also accounts for the expression of antigens by human tumors. 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. The first example is a point mutation in a previously unknown gene, that produces a new antigenic peptide which, remarkably, is partially encoded by the 5′ end of an intron [52]. Another example is a point mutation in the cyclin-dependent kinase 4 gene, which alters the regulation of the activity of this protein and may therefore contribute to oncogenesis [53]. In another melanoma line, an antigenic peptide arises through a point mutation in the β-catenin gene, which may alter the adhesion properties of the tumor cell [54]. Point mutations may also create tumor antigens by directly altering an HLA molecule: autologous CTL directed against a human renal cell carcinoma were recently found to recognize an HLA-A2 molecule that was altered as a result of a point mutation changing one amino acid in the alpha-2 helix [55].

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. However, 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.

Clinical prospects

The next few years will see the evaluation of new clinical trials using some of these defined tumor antigens. Progress at three different levels is anticipated. Firstly, a knowledge of the molecular nature of these antigens allows the selection of patients whose tumor actually expresses a given antigen. Eligible tumors should express the relevant gene along with the appropriate HLA class I specificity, and this can be tested readily by reverse transcriptase/polymerase chain reaction on RNA extracted from a small tumor sample. The fraction of tumors expressing a given antigen can be calculated from the frequency of expression of the relevant gene in that tumor type and from the frequency of the given class I molecule in the population. In Caucasians, about 60% of melanomas express at least one of the tumor-specific shared antigens thus far defined.

Secondly, the definition of the molecular nature of tumor antigens allows the rational design of highly specific vaccine preparations. These could consist of engineered cells expressing the antigens or of antigenic peptides mixed with appropriate adjuvants. The availability of the genes encoding the antigens also allows the preparation of recombinant proteins that can be combined with adjuvants, or the preparation of recombinant viral vectors that can be used for immunization.

Thirdly, the precise knowledge of the antigenic epitopes involved in the immune response of an individual patient against his own tumor cells allows careful monitoring of the CTL responses to these epitopes after the vaccination. This should help to optimize the immunization procedure.

It is currently impossible to predict the clinical outcome of such strategies, but preliminary results with the MAGE-3.A1 peptide are promising [56]. Since a number of tumors appear to express several antigens, patients bearing these tumors could be immunized simultaneously with several distinct defined antigens. This ought to eliminate the tumor cells more effectively. It should also reduce the emer-

gence of antigen-loss variants arising by loss of antigen expression, as it is unlikely that the same variant would simultaneously lose distinct nominal antigens.

References

1. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. J Natl Cancer Inst 1957; 18:769.
2. Rouse BT, Röllinghoff M, Warner NL. Anti-ϑ serum-induced suppression of the cellular transfer of tumour-specific immunity to a syngeneic plasma cell tumour. Nature New Biol 1972; 238:116.
3. Brunner KT, McDonald HR, Cerottini J-C. Quantification and clonal isolation of cytolytic T lymphocyte precursors selectively infiltrating murine sarcoma virus-induced tumors. J Exp Med 1981; 154:362.
4. Anichini A, Fossati G, Parmiani G. Clonal analysis of the cytolytic T-cell response to human tumors. Immunol Today 1987; 8:385.
5. Bruggen P van der, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991; 254:1643.
6. Traversi C, Bruggen P van der, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T. 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 1992; 176:1453.
7. Wölfel T, Van Pel A, Brichard V, Schneider J, Seliger B, Meyer zum Büschenfelde K-H, Boon T. Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. Eur J Immunol 1994; 24:759.
8. Cox AL, Skipper J, Chen Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CL Jr. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science 1994; 264:716.
9. Jung S, Schluesener HJ. Human T lymphocytes recognize a peptide of single point-mutated, oncogenic ras proteins. J Exp Med 1991; 173:273.
10. Celis E, Tsai V, Crimi C, DeMars R, Wentworth PA, Chesnut RW, Grey HM, Sette A, Serra HM. Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes. Proc Natl Acad Sci USA 1994; 91:2105.
11. Bruggen P van der, Bastin J, Gajewski T, Coulie PG, Boël P, De Smet C, Traversari C, Townsend A, Boon T. 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 1994; 24:3038.
12. De Plaen E, Arden K, Traversari C, Gaforio JJ, Szikora J-P, De Smet C, Brasseur F, Bruggen P van der, Lethé B, Lurquin C, Brasseur R, Chomez P, De Backer O, Cavenee W, Boon T. Structure, chromosomal localization and expression of twelve genes of the MAGE family. Immunogenetics 1994; 40:360.
13. Boël P, Wildmann C, Sensi M-L, Brasseur R, Renauld J-C, Coulie P, Boon T, Bruggen P van der. BAGE, a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity 1995; 2:167.
14. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med 1995; 182:689.
15. Brasseur F, Marchand M, Vanwijck R, Hérin M, Lethé B, Chomez P, Boon T. Human gene MAGE-1, which codes for a tumor rejection antigen, is expressed by some breast tumors. Int J Cancer 1992; 52:839.
16. Brasseur F, Rimoldi D, Liénard D, Lethé B, Carrel S, Arienti F, Suter L, Vanwijck R, Bourlond A, Humblet Y, Vacca A, Conese M, Lahaye T, Degiovanni G, Deraemaecker R, Beauduin M, Sastre X, Salamon E, Dréno B, Jäger E, Knuth A, Chevreau C, Suciu S, Lachapelle M, Pouillart P, Parmiani G, Lejeune F, Cerottini J-C, Boon T, Marchand M. Expression of MAGE genes in primary and metastatic cutaneous melanoma. Int J Cancer 1995; 63:375.
17. Weynants P, Lethé B, Brasseur F, Marchand M, Boon T. Expression of MAGE genes by non-small-cell lung carcinomas. Int J Cancer 1994; 56:826.
18. Bruggen P van der, Szikora J-P, Boël P, Wildmann C, Somville M, Sensi M, Boon T. Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing HLA-Cw*1601. Eur J Immunol 1994; 24:2134.
19. Gaugler B, Van den Eynde B, Bruggen P van der, Romero P, Gaforio JJ, De Plaen E, Lethé B, Brasseur F, Boon T. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med 1994; 179:921.
20. Herman J, Bruggen P van der, Luescher I, Mandruzzato S, Romero P, Thonnard J, Fleischhauer K, Boon T, Coulie PG. 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 1996; 43:377.
21. Takahashi K, Shichijo S, Noguchi M, Hirohata M, Itoh K. Identification of MAGE-1 and MAGE-4 proteins in spermatogonia and primary spermatocytes of testis. Cancer Res 1995; 55:3478.
22. Chomez P, Williams R, De Backer O, Boon T, Vennström B. The SMAGE gene family is expressed in post-meiotic spermatids during mouse germ cell differentiation. Immunogenetics 1995; 43:97.
23. Haas GG Jr, D'Cruz OJ, De Bault LE. Distribution of human leukocyte antigen-ABC and -D/DR antigens in the unfixed human testis. Am J Reprod Immunol Microbiol 1988; 18:47.
24. Uyttenhove C, Godfraind C, Lethé B, Amar-Costesec A, Renauld J-C, Gajewski TF, Duffour T, Warnier G, Boon T, Van den Eynde BJ. The expression of mouse gene P1A in testis does not prevent safe induction of cytolytic T cells against a P1A-encoded tumor antigen. Int J Cancer. In Press
25. De Smet C, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T. The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. Proc Natl Acad Sci USA 1996; 93:7149.
26. Guilloux Y, Lucas S, Brichard VG, Van Pel A, Viret C, De Plaen E, Brasseur F, Lethé B, Jotereau F, Boon T. A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas in encoded by an intron sequence of the *N*-acetylglucosaminyltransferase V gene. J Exp Med 1996; 183:1173.
27. Gaugler B, Brouwenstijn N, Vantomme V, Szikora J-P, Van der Spek CW, Patard J-J, Boon T, Schrier P, Van den Eynde BJ. A new gene coding for an antigen recognized by autologous cytolytic T lymphocytes on a human renal carcinoma. Immunogenetics 1996; 44:323.
28. Ressing ME, Sette A, Brandt RMP, Ruppert J, Wentworth PA, Hartman M, Oseroff C, Grey HM, Melief CJM, Kast WM. Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides. J Immunol 1995; 154:5934.
29. Anichini A, Maccalli C, Mortarini R, Salvi S, Mazzocchi A, Squarcina P, Herlyn M, Parmiani G. Melanoma cells and normal melanocytes share antigens recognized by HLA-A2-restricted cytotoxic T cell clones from melanoma patients. J Exp Med 1993:177:989.
30. Brichard V, Van Pel A, Wölfel T, Wölfel C, De Plaen E, Lethé B, Coulie P, Boon T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 1993; 178:489.
31. Coulie PG, Brichard V, Van Pel A, Wölfel T, Schneider J, Traversari C, Mattei S, De Plaen E, Lurquin C, Szikora J-P, Renauld J-C, Boon T. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 1994; 180:35.

32. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA 1994; 91:3515.
33. Bakker ABH, Schreurs MWJ, Boer AJ de, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med 1994; 179:1005.
34. Wang R-F, Robbins PF, Kawakami Y, Kang X-Q, Rosenberg SA. Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. J Exp Med 1995; 181:799.
35. Robbins PF, El-Gamil M, Kawakami Y, Rosenberg SA. Recognition of tyrosinase by tumor-infiltrating lymphocytes from a patient responding to immunotherapy. Cancer Res 1994; 54:3124.
36. Brichard VG, Herman J, Van Pel A, Wildmann C, Gaugler B, Wölfel T, Boon T, Lethé B. A tyrosinase nonapeptide presented by HLA-B44 is recognized on a human melanoma by autologous cytolytic T lymphocytes. Eur J Immunol 1996; 26: 224.
37. Topalian SL, Rivoltini L, Mancini M, Markus NR, Robbins PF, Kawakami Y, Rosenberg SA. Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc Natl Acad Sci USA 1994; 91:9461.
38. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, Yannelli JR, Adema GJ, Miki T, Rosenberg SA. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci USA 1994; 91:6458.
39. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JR, Appella E, Rosenberg SA. 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 1994; 180:347.
40. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, Robbins PF, Sette A, Appella E, Rosenberg SA. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol 1995; 154:3961.
41. Castelli C, Storkus WJ, Maeurer MJ, Martin DM, Huang EC, Pramanik BN, Nagabhushan TL, Parmiani G, Lotze MT. Mass spectrometric identification of a naturally processed melanoma peptide recognized by CD8+ cytotoxic T lymphocytes. J Exp Med 1995; 181:363.
42. Kang X, Kawakami Y, El-Gamil M, Wang R, Sakaguchi K, Yannelli JR, Appella E, Rosenberg SA, Robbins PF. Identification of a tyrosinase epitope recognized by HLA-A24-restricted, tumor-infiltrating lymphocytes. J Immunol 1995; 155:1343.
43. Skipper JCA, Hendrickson RC, Gulden PH, Brichard V, Van Pel A, Chen Y, Shabanowitz J, Wölfel T, Slingluff CL Jr, Boon T, Hunt DF, Engelhard VH. An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins. J Exp Med 1996; 183:527.
44. Topalian SL, Gonzales MI, Parkhurst M, Li YF, Southwood S, Sette A, Rosenberg SA, Robbins PF. Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J Exp Med 1996; 183:1965.
45. Wang R-F, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA. Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J Exp Med 1996; 183:1131.
46. Mattes MJ, Thomson TM, Old LJ, Lloyd KO. A pigmentation-associated, differentiation antigen of human melanoma defined by a precipitating antibody in human serum. Int J Cancer 1983; 32:717.
47. Bystryn J-C, Darrell R, Friedman RJ, Kopf A. Prognostic significance of hypopigmentation in malignant melanoma. Arch Dermatol 1987; 123:1053.
48. Richards JM, Mehta N, Ramming K, Skosey P. Sequential chemoimmunotherapy in the treatment of metastatic melanoma. J Clin Oncol 1992; 10:1338.
49. Boon T. Toward a genetic analysis of tumor rejection antigens. Adv Cancer Res 1992; 58:177.
50. Mandelboim O, Berke G, Fridkin M, Feldman M, Eisenstein M, Eisenbach L. CTL induction by a tumour-associated antigen octapeptide derived from a murine lung carcinoma. Nature 1994; 369:67.
51. Monach PA, Meredith SC, Siegel CT, Schreiber H. A unique tumor antigen produced by a single amino acid substitution. Immunity 1995; 2:45.
52. Coulie PG, Lehmann F, Lethé B, Herman J, Lurquin C, Andrawiss M, Boon T. A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc Natl Acad Sci USA 1995; 92:7976.
53. Wölfel T, Hauer M, Schneider J, Serrano M, Wölfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer zum Büschenfelde K-H, Beach D. A $p16^{INK4a}$-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 1995; 269:1281.
54. Robbins PF, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E, Rosenberg SA. A mutated β-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med 1996; 183:1185.
55. Brändle D, Brasseur F, Weynants P, Boon T, Van den Eynde B. A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma. J Exp Med 1996; 183:2501.
56. Marchand M, Weynants P, Rankin E, Arienti F, Belli F, Parmiani G, Cascinelli N, Bourlond A, Vanwijck R, Humblet Y, Canon J-L, Laurent C, Naeyaert J-M, Plagne R, Deraemaeker R, Knuth A, Jäger E, Brasseur F, Herman J, Coulie PG, Boon T. Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. Int J Cancer 1995; 63:883.