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Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor

(tumor antigen/immunotherapy/HLA-A2/melanocyte/MART-1)

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ABSTRACT **By cDNA expression cloning we have isolated a gene encoding a shared human melanoma antigen recognized by HLA-A2 restricted autologous and allogeneic tumor-infiltrating lymphocytes (TILs) from patients with metastatic melanoma. By using both transient and stable expression systems, transfection of this gene into non-antigen-expressing HLA-A2$^+$ cell lines resulted in recognition by the antigen-specific TILs. The sequence of this cDNA revealed a previously undescribed putative transmembrane protein whose expression was restricted to melanoma and melanocyte cell lines and human retina but no other fresh or cultured normal tissues tested or other tumor histologies. Thus, we have identified a gene encoding a melanocyte lineage-specific protein (MART-1; melanoma antigen recognized by T cells 1) that is a widely shared melanoma antigen recognized by the T lymphocytes of patients with established malignancy. Identification of this gene opens possibilities for the development of immunotherapies for patients with melanoma.**

The existence of lymphocytes within melanoma deposits that, when isolated, are capable of recognizing specific tumor antigens on autologous and allogeneic melanomas in a major histocompatibility complex (MHC) restricted fashion provides strong evidence that an immune response to cancer exists in humans (1–8). The ability of these tumor-infiltrating lymphocytes (TILs) to mediate the regression of cancer when adoptively transferred into patients with metastatic melanoma attests to the clinical importance of the antigens recognized (9, 10). Characterization of these antigens may thus be important for development of strategies for cancer immunotherapy.

van der Bruggen and coworkers and Brichard *et al.* (11–13) have characterized two genes coding for melanoma antigens, MAGE-1 and tyrosinase, by using T-cell clones established from the peripheral blood of patients who were repetitively immunized *in vivo* with mutagenized tumor cells or whose peripheral blood lymphocytes were sensitized by repetitive *in vitro* stimulation with tumor. The strategy used in the present study attempted to identify the genes coding for tumor antigens that were recognized by TILs from tumor-bearing patients in the absence of immunization to enhance the possibility that genes would be identified that coded for antigens involved in the natural immune response against the growing cancer. Anti-melanoma T cells appear to be enriched in TILs probably as a consequence of clonal expansion and accumulation at the tumor site *in vivo* (14). We have now cloned and sequenced a gene coding for a shared, commonly expressed melanoma antigen, restricted by HLA-A2.1.§

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MATERIALS AND METHODS

Generation of Cytotoxic T Lymphocytes (CTLs) and Culture of Cell Lines. CTLs were generated from excised tumor specimens by culturing a suspension of cells with interleukin 2 (IL-2) (6000 units/ml) (Chiron) for 30–70 days as reported (15). TIL501 and TIL1235 were predominantly CD8$^+$ and were derived from the tumor specimens of patients with advanced metastatic melanoma. The CD8$^+$ T-cell clone TIL501.A42 was established by limiting dilution methods and cultured with IL-2 (120 units/ml) plus irradiated autologous tumor cells.

Melanoma cell lines (397mel, 501mel, 526mel, 537mel, 624mel, 888mel, and 952mel) and Epstein–Barr virus transformed B-cell lines (501EBVB and 836EBVB) were established in our laboratory and cultured in RPMI 1640 medium containing 10% fetal calf serum (FCS). Normal cultured melanocytes (NHEM483, NHEM493, NHEM527, NHEM529, NHEM530, NHEM533, NHEM616, and NHEM680) were purchased from Clonetics (San Diego); FM725, FM801, and FM902 were provided by M. Herlyn (Wistar Institute, Philadelphia); and HA002 was provided by R. Halaban (Yale University, New Haven, CT) and was cultured in melanocyte growth medium (MGM; Clonetics). Melanoma cell lines C32, RPMI7951, WM115, A375, HS695T, and Malme3M; colon cancer cell lines Collo, SW480, and WiDr; breast cancer cell lines MDA231, MCF7, HS578, and ZR75; neuroblastoma cell line SK-N-SH; glioma cell lines U138MG, HS683, and H4; sarcoma cell line 143B; and embryonal kidney cell line 293 transformed with adenovirus type 5 were purchased from American Type Culture Collection. Renal cancer cell lines UOK108 and UOK117 were provided by M. Linehan (National Institutes of Health). The small cell lung cancer cell line H1092 was provided by J. D. Minna (University of Texas Southwestern Medical Center, Dallas). Ewing's sarcoma cell lines TC71, RD-ES, and 6647 were provided by M. Tsokos (National Institutes of Health). The neuroblastoma cell line SK-N-AS was provided by O. M. El Badry (National Institutes of Health). The plasmacytoma cell line HMY-C1R and the M1 fibroblast cell line were provided by W. Biddison (National Institutes of Health). Kidney epithelial cells KAM and WLC were provided by D. J. Hazen-Martin and D. A. Sens (Medical University of South Carolina, Charleston). The monkey kidney cell line COS-7 was provided by W. Leonard (National Institutes of Health).

Cytotoxicity Assay. ^{51}Cr release assays were performed as reported (15). Briefly, 5000 target cells labeled with ^{51}Cr were mixed with various numbers of effector cells and incubated

Abbreviations: CTL, cytotoxic T lymphocyte; IFN-γ, interferon γ; IL-2, interleukin 2; TIL, tumor-infiltrating lymphocyte; MHC, major histocompatibility complex.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U06452).

for 5 h. Then supernatants were collected, radioactivity was measured, and percentage specific lysis was calculated.

Interferon γ (IFN-γ) Release Assay. Responder cells (50–100 × 10^3) and stimulator cells (4 × 10^4–10^5) were mixed in 300 μl of AIM-V medium containing IL-2 (120 units/ml per well) in a 96-flat well microplate. After incubation for 20 h, 100 μl of supernatant was collected and added to an ELISA plate (Immunoplate MaxiSorp, Nunc) coated with anti-human IFN-γ monoclonal antibody (Biosource, Camerillo, CA). After overnight incubation at 4°C, the plates were washed three times and 100 μl of a 1:2000 dilution of rabbit anti-human IFN-γ polyclonal antibody (Biosource) was added and incubated at 37°C for 2 h. Plates were washed three times, and 100 μl of a 1:2000 dilution of alkaline phosphatase-labeled goat anti-rabbit IgG polyclonal antibody (Boehringer Mannheim) was added. After a 1-h incubation at 37°C, 100 μl of *p*-nitrophenyl phosphate (4 mg/ml) (Sigma) was added and incubated for 10–20 min at room temperature in the dark; 25 μl of 1M NaOH was added to stop the reaction. Optical density was measured at 405 nm and the concentration of IFN-γ was calculated in comparison to recombinant IFN-γ standards (Biogen) measured in the same assay.

cDNA Expression Cloning. A cDNA library was constructed from poly(A) RNA from the HLA-A2$^+$ melanoma cell line 501mel as described (16, 17). Briefly, first-strand cDNA was synthesized with a linker primer GGACAGGCCGAGGCGGCC$(T)_{40}$ followed by second-strand cDNA synthesis. After treatment with T4 DNA ligase, an *Sfi* I adaptor consisting of two oligonucleotides, CCAATCGCGACC and GGTCGCGATTGGTAA, was ligated to the end of the cDNA. The cDNA was digested with *Sfi* I and the digested fragment was isolated by passing through a spun column. The cDNA was then mixed with bacteriophage λpCEV27 vector arms prepared by *Sfi* I digestion and *in vitro* packaging was performed.

To screen for melanoma antigens, 10 μg of the amplified cDNA library containing ≈10^7 clones was transfected into the HLA-A2$^+$ antigen-non-expressing cell lines MDA231 clone 7 and A375 clone 1–4 by a modified calcium phosphate method (Mammalian Transfection Kit, Stratagene). After G418 (BRL) selection, individual colonies were isolated and cultured in 96-well microplates and replica plates were made. A mixture of 5 × 10^4 TIL1200 and 5 × 10^4 TIL1235 was added to the wells of the microplates containing the growing transfectants that were near confluence and incubated for 20 h. Supernatants were collected and IFN-γ was measured by ELISA.

PCR was performed to rescue the transfected genes from the genomic DNA of positive transfectants by using SP6 and T7 primers, which flank the inserted genes. The amplified products were cloned in the pCRII vector (Invitrogen, San Diego). For cDNA clones 22 and 23, a *Hin*dIII/*Xho* I fragment containing the full-length cDNA was subcloned into the expression vector pcDNA3 (Invitrogen).

To test whether the cloned cDNAs encode tumor antigens, the pcDNA3 containing the cloned genes was transiently transfected into the COS-7 cell line by the DEAE-dextran method (18). Briefly, 3 × 10^5 cells per well in 6-well plates were incubated at 37°C for 4 h in 0.75 ml of Dulbecco's modified Eagle's medium (DMEM) containing 100 μg of DEAE-dextran (Sigma), 0.1 mM chloroquine, and 1 μg of the pcDNA3 containing the cloned genes and/or the pcDNA3-HLA-A2.1. After medium was removed, 10% dimethyl sulfoxide solution in Hanks' balanced salt solution buffer was added and incubated for 2 min. The cells were washed once with PBS and incubated in 7.5% FCS DMEM for 2 days. The 293 cell line was transiently transfected by using Lipofectamine (BRL) according to the manufacturer's recommendation. After incubation, the ability of the transfected COS-7 or 293 cells to mediate IFN-γ release from TILs was assessed. Expression of the *HLA-A2* gene was tested by flow cytometry. Stable transfectants were made by the calcium phosphate method and individual colonies and pooled transfectants were tested for reactivity to TILs by cytotoxicity and IFN-γ release assays.

DNA sequencing of the cloned genes was performed by the dideoxynucleotide chain-termination method with dGTP and 7-deaza-dGTP. DNA and protein sequences were analyzed by the Genetics Computer Group (Madison, WI) program with GenBank and EMBL data bases and Swiss-Prot, Protein Identification Resource, GenPept, and Brookhaven Protein Data Bank protein data bases.

Northern Blot Analysis. Total RNA was isolated by the guanidine isothiocyanate/cesium chloride centrifugation method. Total RNA from normal tissue was purchased from Clontech. Total RNA (10–20 μg) was subjected to electrophoresis in a 1% agarose formaldehyde gel and transferred to a nylon membrane (Duralon-UV membranes; Stratagene). The *Sal* I-digested fragment containing the full-length cDNA from clone 22 and the β-actin cDNA (Clontech) were labeled by random priming and used as a probe. Hybridization with the probe was performed according to the QuikHyb protocol (Stratagene) at 68°C for 2–16 h. Membranes were washed two times with 2× standard saline citrate (SSC)/0.1% SDS at 60°C for 15 min and once with 0.1× SSC at 60°C for 30 min, and then autoradiography was performed.

RESULTS

Characterization of Cultured TILs from Melanoma Patients. Multiple TIL lines were established from HLA-A2$^+$ melanoma patients and tested for lysis of melanoma cell lines from HLA-A2$^+$ and HLA-A2$^-$ patients. HLA-A2 was selected because it is the most frequently expressed class I MHC antigen (≈50% of individuals) and has been shown to be a dominant restriction element for recognition of melanoma antigens (19). TIL501, TIL1235, and TIL1200 exhibited specific recognition of shared melanoma antigens in an HLA-A2 restricted fashion. TIL501.A42 was a T-cell clone established from TIL501 by limiting dilution. These TILs caused lysis or released cytokines including IFN-γ, tumor necrosis factor α, and granulocyte–macrophage colony-stimulating factor when cocultured with a variety of HLA-A2$^+$ melanoma or melanocyte cell lines but not HLA-A2$^-$ melanoma lines or HLA-A2$^+$ nonmelanoma cell lines including the breast cancer cell line MDA231. Two representative experiments are shown in Table 1. Thus, these CTLs seemed to recognize a nonmutated peptide derived from a melanocyte lineage-specific antigen.

Cloning of cDNA Coding for Melanoma Antigens Recognized by T Cells. A cDNA library from the HLA-A2$^+$ 501mel melanoma cell line was transfected into two highly transfectable HLA-A2.1$^+$ cancer cell lines, MDA231 and A375. These cell lines were not lysed by melanoma-specific TILs but were lysed by HLA-A2 restricted influenza M1-specific CTLs after incubation with the $M1_{58-66}$ peptide or infection with a recombinant vaccinia virus containing the M1 gene (data not shown). Thus, these cell lines exhibited normal antigen processing and presenting ability but were not lysed by these melanoma-specific TILs because of the lack of expression of the relevant melanoma antigens. After selection with G418, ≈6700 transfected clones from each cell line were isolated and grown in microplates. Using the IFN-γ release assay, 21 MDA231 and 27 A375 positive clones were isolated and rescreened. Of these clones, 8 MDA231 and 7 A375 clones were positive in a second screening assay.

To rescue the integrated genes, PCR using genomic DNA from these positive transfectants was performed with SP6 and T7 primers flanking the insert genes. Eight genes that were amplified from the seven transfectants that showed one

Table 1. Specificity of TIL501.A42 and TIL1235 for melanomas and melanocytes

Lytic specificity					Cytokine release specificity				
		% lysis					IFN-γ, pg/ml		
Target*	HLA-A2	TIL501.A42	TIL1235	LAK cells	Stimulator†	HLA-A2	TIL501.A42	TIL1235	TIL586
501mel	+	54	51	78	501mel	+	647	219	0
526mel	+	25	33	74	586mel	−	0	0	1034
624mel	+	23	27	75	NHEM493	+	1835	850	0
952mel	+	10	11	75	NHEM527	+	1638	749	0
Malme3M	+	36	41	70	NHEM530	+	1224	2532	0
C32	+	17	23	82	NHEM533	+	300	251	0
RPMI7951	+	1	6	67	NHEM616	+	635	423	0
WM115	+	−2	3	68	FM725	+	5975	1471	0
HS695T	+	1	2	87	FM801	+	1375	893	62
397mel	−	−1	0	70	NHEM483	−	0	0	0
MDA231	+	0	3	94	NHEM680	−	0	0	548
					HA002	−	0	0	0

TIL501.A42 and TIL1235 lysed most HLA-A2 melanoma cell lines and secreted IFN-γ when cultured with HLA-A2 melanomas and melanocytes. LAK, lymphokine-activated killer cells. TIL586 is a class I MHC restricted melanoma-specific TIL not restricted by HLA-A2. TIL586 shares HLA-A29, -A31, and -B44 with NHEM680

*^{51}Cr release assay was performed at an effector/target cell ratio of 20:1 for TIL501.A42 and of 40:1 for TIL1235. All targets were melanoma cell lines except for MDA231, which is a breast cancer cell line.

†IFN-γ in the supernatant was measured after TILs and stimulator cells were coincubated for 20 h. IFN-γ secreted by TILs alone without stimulators (<50 pg/ml) was subtracted. 501mel and 586mel are melanoma cell lines. All others were normal melanocyte cell lines.

or two sharp bands, including a 1.6-kb band from MDA-22 and MDA-23 transfectants, were subcloned into the pCRII cloning vector and then further cloned into the pcDNA3 eukaryotic expression vector. The 1.6-kb band detected by Northern blot analysis with the cDNA 22 probe suggested that this fragment was a full-length cDNA.

Transient transfection of the expression vector pcDNA3 containing the cDNA from clone 22 or 23 into either COS-7 or 293 cells along with the *HLA-A2.1* gene conferred reactivity to TIL1235 and TIL501.A42 as demonstrated by the specific release of IFN-γ (Table 2, Exps. 1 and 2). Stable transfection of these cDNA fragments into MDA231 or A375mel cell lines also conferred reactivity to TIL1235 and TIL501.42 (Table 2, Exp. 3). TIL501.A42 could lyse MDA231 stably transfected with cDNA 22 (data not shown). These results indicated that these cDNAs encode a melanoma antigen recognized by HLA-A2 restricted TILs from melanoma patients. Transfection of another clone, MDA-25, stimulated release of IFN-γ only from TIL1200. Characterization of this cDNA revealed it to be almost identical to a previously described melanoma antigen (gp100) recognized by monoclonal antibody HMB45 and will be described in more detail elsewhere (37).

Table 2. IFN-γ secretion by TIL501.A42 and TIL1235 when cultured with HLA-A2$^+$ cell lines transfected with gene 22 or 23

Stimulator cells	Transfected gene	HLA-A2	IFN-γ, pg/ml TIL501.A42	IFN-γ, pg/ml TIL1235
		Experiment 1		
501mel	None	+	1009	1076
397mel	None	−	0	0
COS7	None	−	0	0
COS7	*HLA-A2.1*	+	0	0
COS7	22	−	0	0
COS7	*HLA-A2.1+22*	+	771	1049
		Experiment 2		
501mel	None	+	ND	1051
397mel	None	−	ND	0
293	*HLA-A2.1*	+	ND	0
293	22	−	ND	0
293	*HLA-A2.1+22*	+	ND	255
		Experiment 3		
501mel	None	+	1073	1056
397mel	None	−	0	0
MDA231	None	+	0	0
MDA231	23	+	674	725
A375	None	+	0	0
A375	23	+	264	131

IFN-γ in the supernatant was measured after TILs were coincubated for 20 h with COS-7 or 293 cell lines transiently transfected with the pcDNA3 containing the HLA-A2.1 and/or cDNA 22 by the DEAE-dextran method (Exps. 1 and 2), or with the A375 or MDA231 cell lines stably transfected with cDNA 23 (Exp. 3). IFN-γ was secreted only when TILs were incubated with HLA-A2$^+$ cell lines transfected with cDNA 22 or 23. IFN-γ secreted by TILs alone without stimulator (<50 pg/ml) was subtracted. ND, not done.

The cDNA sequences of clones 22 and 23 were identical except at a single base that was believed to be a change introduced by PCR. Two other independently amplified fragments were also sequenced to clarify this region and the consensus sequence is shown in Fig. 1. The longest open reading frame in this gene consists of 354 bases corresponding to a 118-amino acid protein of 13 kDa. This sequence did not show significant similarity to any complete nucleotide or protein sequences in established data bases. Amino acids 27–47 consist of a hydrophobic region that may contain the HLA-A2 binding peptides (20–23). We have called the antigen encoded by cDNA 22 and 23 the MART-1 antigen (melanoma antigen recognized by T cells 1). Of the 10 HLA-A2 restricted TIL lines generated in our laboratory, 9 recognized MART-1, 4 recognized gp100, and none appeared to recognize MAGE-1 or tyrosinase (ref. 24; data not shown).

Expression of MART-1. Northern blot analysis of a variety of cell lines including melanoma, melanocyte, and nonmelanoma cancer cell lines and normal tissues was performed to evaluate the expression of the gene coding for MART-1 (Table 3). Seven of 11 HLA-A2$^+$ melanoma cell lines, all 4 HLA-A2$^-$ melanoma cell lines, and all 7 melanocyte cell lines tested were positive for MART-1 RNA expression, although expression in melanocytes was less than that in melanomas. In this Northern blot analysis, all HLA-A2$^+$ melanoma cell lines recently established in our laboratory expressed MART-1 RNA. There was a perfect correlation between MART-1 expression and lysis by TIL501.A42 in the 10 HLA-A2$^+$ melanoma lines shown in Table 3. TIL501.A42, which recognized the MART-1 antigen, lysed 13 of 17 (76%) HLA-A2$^+$ melanoma cell lines tested (unpublished data). Of

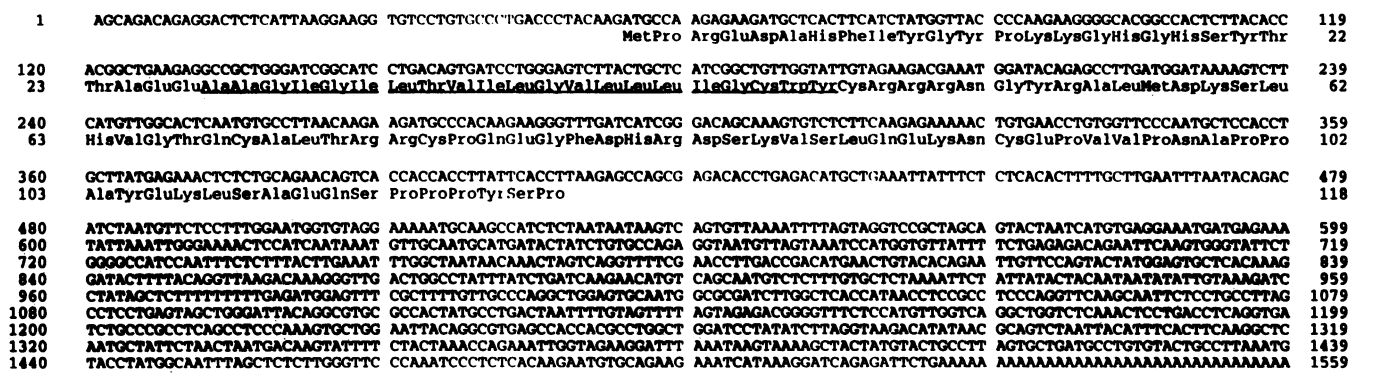

FIG. 1. Nucleotide and predicted amino acid sequence of the cDNA encoding the MART-1 antigen. Hydrophobic region is underlined.

10 normal human tissues examined for mRNA expression by Northern blot analysis, only retina was positive. No positivity was seen in any cell lines from T cells, B cells, kidney epithelial cells, or fibroblasts or in 19 nonmelanoma tumors. It thus appears that MART-1 is a previously undescribed antigen expressed on melanocyte lineage cells from skin and retina that is also expressed on melanoma cells.

DISCUSSION

T cells play an important role in tumor regression in most murine tumor models. TILs that recognize unique cancer antigens can be isolated from many murine tumors and the adoptive transfer of these TILs plus IL-2 can mediate the regression of established lung and liver metastases (25). The secretion of IFN-γ by injected TILs significantly correlated with *in vivo* regression of murine tumors (26).

TILs from patients with metastatic melanoma recognize shared antigens including melanocyte–melanoma lineage-specific tissue antigens *in vitro* (27, 28). These TILs appear to recognize nonmutated self peptides since they also recognize normal cultured melanocytes. The known ability of TIL administration to mediate the regression of metastatic cancer in 35–40% of melanoma patients suggests that the antigens recognized by TILs play an important role in the immune response against growing melanomas (9, 10). We thus attempted to use these TILs to clone the genes encoding these melanocyte–melanoma antigens to enhance the possibility of identifying antigens of value in the immunotherapy of patients with melanoma.

Studies using a panel of T-cell clones and immunoselected melanoma clones (29, 30), as well as studies analyzing HPLC fractionated peptides from melanoma cells (31, 32), suggest that multiple antigenic peptides that can provoke an immune response exist on melanomas. By cDNA cloning, we have now identified two antigens, MART-1 and gp100, that are recognized by HLA-A2.1 restricted TILs. The MART-1 antigen described in detail in this paper is a 118-amino acid protein of 13 kDa. Neither the gene nor the amino acid sequence has been previously described and the function of this protein is unknown. The MART-1 antigen contains a highly hydrophobic region from amino acids 27 to 47 followed by three arginine residues, suggestive of a transmembrane protein. Although no significant homology exists to the entire protein there is a 27-amino acid segment (amino acids 57–83) that is 37% identical to a type II membrane protein previously

Table 3. Expression of the MART-1-encoding gene in a variety of tissue and cell lines

Melanoma		Normal fresh tissue		Colon cancer	
HLA-A2$^+$		Brain	−	Collo	−
501 mel		Retina	+	SW480	−
526mel	+	Adrenal gland	−	WiDr	−
624mel	+	Lung	−	Breast cancer	
Malme3M	+	Liver	−	MDA231	−
952mel	+	Kidney	−	MCF7	−
697mel	+	Spleen	−	HS578	−
C32	+	Thymus	−	ZR75	−
RPMI7951	+	Testis	−	Neuroblastoma	
HS695T	−	Fetal liver	−	SK-N-AS	−
WM115	−	Nonmelanoma cell lines		SK-N-SH	−
A375	−	T-cell		Ewing sarcoma	
HLA-A2$^-$	−	TILA	−	TC75	−
397mel	+	TILB	−	RD-ES	−
888mel	+	B cell		6647	−
537mel	+	Daudi	−	Sarcoma	
586mel	+	HMY-C1R	−	143B	−
Melanocyte		501EBVB	−	Glioma	
NHEM483	+	836EBVB	−	U138MG	−
NHEM493	+	Fibroblast		HS683	−
NHEM529	+	M1 fibroblast	−	Renal cell cancer	
NHEM530	+	Kidney epithelial cell		UOK108	−
FM902	+	KAM	−	UOK117	−
FM906	+	WLC	−	Small cell lung cancer	
HA002	+			H1092	−

Northern blot analysis with 10–20 μg of total RNA was probed with the full-length cDNA of gene 22. RNAs from most melanomas, all melanocyte cell lines tested, and retina were positive.

recognized as mouse natural killer cell surface protein NKR-P1 (33). MART-1 does not contain a leader sequence characteristic of many type I membrane proteins.

MART-1 RNA was expressed in 11 of 14 HLA-A2.1$^+$ or HLA-A2.1$^-$ melanoma lines and in 7 of 7 melanocyte lines. We do not yet know whether restriction elements other than HLA-A2.1 can present the MART-1 antigen. With the exception of retinal tissue, no MART-1 expression was found on any normal tissue tested, T-cell lines, B-cell lines, kidney epithelial lines, a fibroblast line, or 19 tumor cell lines from cancers of the colon, breast, brain, kidney, lung, or bone.

Two other antigens, MAGE-1 and tyrosinase, have been described that are recognized by T cells derived from peripheral blood lymphocytes after repetitive *in vivo* or *in vitro* immunization (11–13). MAGE-1 is expressed on a variety of cancer cells and testis. Tyrosinase appears to be a melanocyte tissue-specific antigen. However, none of our 10 HLA-A2 restricted TILs appeared to recognize MAGE-1 or tyrosinase. This may be attributed to the different methods used to generate anti-MAGE or anti-tyrosinase CTLs or a difference in the T-cell repertoire between peripheral lymphocytes and TILs.

The means by which T cells break tolerance to the MART-1 self antigen raise important questions about the nature of the immune response to self antigens on growing cancers. T cells capable of recognizing melanoma–melanocyte antigens can be enriched at sites of growing tumor (14). Overexpression of antigens on the tumor surface in the context of class I MHC and the conditions that exist at sites of inflammation might induce T-cell responses to self antigens that otherwise induce anergy.

The identification of genes associated with melanoma tumor antigens opens possibilities for active specific immunization approaches to the immunotherapy of patients with cancer based on the introduction of these genes into viral or bacterial vector systems. The possibility exists, however, that immune reactions induced against melanocyte–melanoma lineage antigens such as MART-1 may be generated against normal cells. Vitiligo, probably resulting from anti-melanocyte immune reactions, has been reported to be associated with a favorable prognosis in patients with melanoma (34, 35) and has also been reported in patients responding to chemoimmunotherapy (36). We have administered TILs with anti-melanocyte–melanoma reactivities to patients with advanced melanoma (9, 10), and although we have seen sporadic vitiligo in these patients we have not observed any adverse ophthalmologic effects related to the possible expression of these melanocyte antigens on retinal cells.

Because HLA-A2 is present in ≈50% of individuals and the HLA-A2 restricted MART-1 antigen also appears to be widely expressed on melanomas, immunization with the MART-1 antigen may be particularly useful for the development of active immunotherapies.

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