Jpn. J. Cancer Res. 82, 841–847, July 1991

Characterization of the Individual and Cross-reactive Antigens Involved in the Anti-tumor Immunity Induced by Use of an *H-2K-erbB* Recombinant Gene Transfectant

Lin-na Ding,[1] Tomoaki Yoshida,[1] Ken-Ichi Isobe,[1] S. M. Jamshedur Rahman,[1] Fumihiko Nagase,[2] Takashi Yokochi,[3] Kohei Kawashima[4] and Izumi Nakashima[1,5]

Departments of [1]Immunology and [4]Clinical Laboratory, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466, [2]Department of Medical Technology, Nagoya University College of Medical Technology, 1-1-20 Daikominami, Higashi-ku, Nagoya, Aichi 461 and [3]Department of Bacteriology, Aichi Medical University, 21 Karimata, Nagakute, Aichi 480-11

The specificities of the antisera raised in the CDF_1 mice that had been immunized with the P1.HTR tumor cells xenogenized by transfection with recombinant *H-2K^b-erbB* gene were studied. The antisera cross-reacted with a broad range of tumor cell lines maintained either *in vitro* or *in vivo* in an immunofluorescence assay. However, they did not react at all with syngeneic normal tissue cells from thymus, spleen, bone marrow and fetal liver. Even though antigens related to the murine leukemia virus and murine mammary tumor virus (MuMTV) were demonstrated in many of the tumor cell lines tested with specific antibodies, these antigens did not seem to be primarily involved in the anti-P1.HTR antibody activity. The 74 kDa molecule, which was precipitated by the anti-P1.HTR antiserum from the surface radiolabeled cell extract of P1.HTR tumor and was discriminated from the 70 kDa molecule precipitated by the anti-MuMTV serum, was widely distributed among various tumor cell lines tested, but was absent in normal tissue cells. In contrast to the extensive cross-reaction by the antibody, the cytotoxic T lymphocyte generated in the P1.HTR immune mice were shown to be specific to the P1.HTR tumor, and the 98 kDa molecule was precipitated by the anti-P1.HTR serum from the P1.HTR tumor but not from other tumors tested. It is suggested from these results that the 98 kDa molecule is a candidate for an individual tumor-specific transplantation antigen, and is immunodominant for inducing cytotoxic T lymphocytes to coexisting intrinsic retroviral antigens and other serologically cross-reactive tumor antigens.

Key words: *H-2/erbB* recombinant gene transfectant — Individual tumor-specific transplantation antigen — Cross-reactive tumor antigen — Retroviral antigen — Immunodominance

A number of reports have suggested the existence of different types of tumor antigens or tumor-associated antigens which may or may not be involved in tumor rejection.[1,2] They include tumor-associated transplantation antigens (TATA) that are specific to individual chemically induced tumors,[2-4] cross-reactive tumor antigens in virally induced tumors[1] and oncofetal antigens.[5] Both antibody activity and cytotoxic T lymphocyte (CTL) activity against these antigens have been demonstrated. Various strains of mice and tumor cell lines derived from them carry murine leukemia virus (MuLV) and mammary tumor virus (MuMTV).[1] Therefore, antigens related to these viruses must always be taken into account in making an analysis of tumor antigens.[6] Recently we developed a system in which a definite transplantation immunity for tumor rejection is induced by immunizing mice with tumor cells xenogenized through transfection with a hybrid gene between *H-2K^b* and *v-erbB*.[7,8] In this system a high level of antibody activity reacting with the parental tumor cells was developed in parallel with a strong transplantation CTL immunity for tumor rejection. The antibody precipitated 40 kDa, 74 kDa, and 98 kDa molecules from the P1.HTR tumor lysate, and some of these molecules were suspected to be related to retroviral antigens.[2] In the present study, we tried to characterize further the antigens involved in the unique anti-tumor immunity. The results show that the demonstrated anti-tumor antibodies are directed toward antigens which are distributed widely in many tumor cell lines and are distinguished from the known retroviral antigens existing on tumor cells, and suggest that one of the three demonstrated antigens, possibly the 98 kDa molecule is a candidate for an individual tumor-specific TATA which is immunodominant to the intrinsically carried retroviral antigens and other widely distributed, serologically detectable tumor antigens.

MATERIALS AND METHODS

Mice Inbred female (BALB/c×DBA/2) F_1 (CDF_1, *H-2^d*) mice were purchased from Shizuoka Agricultural

[5] To whom correspondence should be addressed.

Center, Hamamatsu, Shizuoka. BALB/c(*H-2*^{d}) and DBA/2(*H-2*^{d}) mice were supplied by the Research Institute for Animal Research, Nagoya University School of Medicine, or were bred in our own laboratory.

Cell lines Table I shows the name, type and origin of the cell lines used in this study. These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 200 m*M* glutamine, 100 U/ml penicillin and 100 U/ml streptomycin (complete RPMI medium). MuMTV-73 tumor cells were maintained in this culture medium containing 2 μg of dexamethasone and 10 μg of insulin. Some tumor cell lines were also maintained by intraperitoneal passages in syngeneic mice.

Transfection Recombinant *H-2-erbB* gene, in which the coding region for a large part of 1 and 2 extracellular domains was replaced with a partial avian erythroblastosis virus *erbB* gene segment encoding the kinase domain, was constructed and transfected into a murine mastocytoma cell line P1.HTR as described previously.[7, 8] The established transfectant and parental P1.HTR cells were used to immunize CDF_1 mice for generation of CTL and production of antiserum.[1, 2]

Preparation of tissue cell suspension Single cell suspensions were prepared from spleen, thymus and fetal liver of mice. Bone marrow cells were collected by flushing out two pieces of femur shafts.

Antibodies The anti-P1.HTR serum was prepared by hyperimmunizing CDF_1 mice with H-2K-erbB transfectant and P1.HTR tumor as described.[7] Anti-MuLV-related G_{IX} antigen (anti-G_{IX}) rabbit antibody[9] was kindly provided by Dr. Old, Memorial Sloan-Kettering Cancer Center, NY; and anti-MuMTV rat antibody[10] was from Dr. Saga, Department of Pathology, Nagoya University School of Medicine. Anti-H-2D^d monoclonal antibody was purchased from Meiji Institute of Health Science, Tokyo.

Flow cytometry analysis Cells were treated with anti-P1.HTR mouse serum (1:200 dilution), anti-G_{IX} rabbit antibody (1:300) or anti-MuMTV rat antibody (1:50) for 30 min at 4°C and then stained with fluorescein-conjugated goat-anti-mouse IgG, goat-anti-rabbit IgG or goat-anti-rat IgG antibodies (1:40, Tago, Inc., Burlingham, CA). Normal mouse serum (1:200) was used as control. Fluorescence intensity for 5000 cells was analyzed by cytofluorography and expressed on a log scale by using a laser flow cytometer (EPICS Profile, Coulter Electronics, Inc., Hialeah, FL). Mean fluorescence intensity (MFI) (arbitrary unit) of the 5000 cells was calculated.

Immunoprecipitation Tumor cells were surface labeled by the ^{125}I-lactoperoxidase method[11] and then lysed in 0.5% Nonidet P-40 detergent in 0.15 *M* NaCl, 0.05 *M*

Table I. Distribution of the Antigens Reactive to Anti-P1.HTR Serum

Cell line	Type[a]	Origin	H-2 haplotype	MFI[b]
P1.HTR	Mastocytoma	DBA/2	d	56
P1.HTR	Mastocytoma (maintained *in vivo*)	DBA/2	d	50
P815	Mastocytoma	DBA/2	d	61
MethA	Fibrosarcoma	BALB/c	d	148
MethA	Fibrosarcoma (maintained *in vivo*)	BALB/c	d	105
L5178Y	Lymphoma	DBA/2	d	160*
P388	Lymphoma	DBA/2	d	77
L1210	B cell-like leukemia	DBA/2	d	48
W231	B cell lymphoma	BALB/c	d	15*
NS-1	Myeloma	BALB/c	d	5.7
EL-4	Thymoma	C57BL/6	b	93
BW5147	Thymoma	AKR/J	k	80
Yac-1	Thymoma	A/Sn	a	69
Normal mouse	Thymocyte	DBA/2	d	1.1*
	Bone marrow cell	DBA/2	d	1.1*
	Spleen cell	DBA/2	d	0.9*
	Liver cell	DBA/2	d	1.0*

a) All tumor cell lines were maintained *in vitro* except where otherwise noted.
b) The means of the MFI values for 2–5 experiments are presented, except for the cases marked *, where the value for one experiment is shown.

Tris and 5 m*M* EDTA at pH 8.0.[12] The cell lysates were immunoprecipitated with test antibodies and *Staphylococcus aureus* protein A. The immunoprecipitated radiolabeled proteins were analyzed by polyacrylamide gel electrophoresis under reducing conditions. Radiolabeled bands in the gel were identified by autoradiography.

Assay for CTL activity For generation of tumor-specific CTL, responder CDF_1 spleen cells (10^7 cells) from P1.HTR-hyperimmune mice plus mitomycin-treated P1.HTR tumor cells (10^6 cells) as the stimulator were cultured in a 24-well plate containing 2 ml/well of complete RPMI 1640 medium for 4 days at 37°C in an atmosphere of 5% CO_2 in air. The CTL activity of these cells was assayed by using the ^{51}Cr-release test as described.[13] Various lines of tumor cells were used as the target. The percentage of specific ^{51}Cr-release was calculated as: (experimental release − spontaneous release)/(total release − spontaneous release) × 100. Total releasable radioactivity was determined from the 1% NP-40 lysate of target cells. Spontaneous release was determined from the culture containing target cells alone. Means of values in triplicate assays are presented.

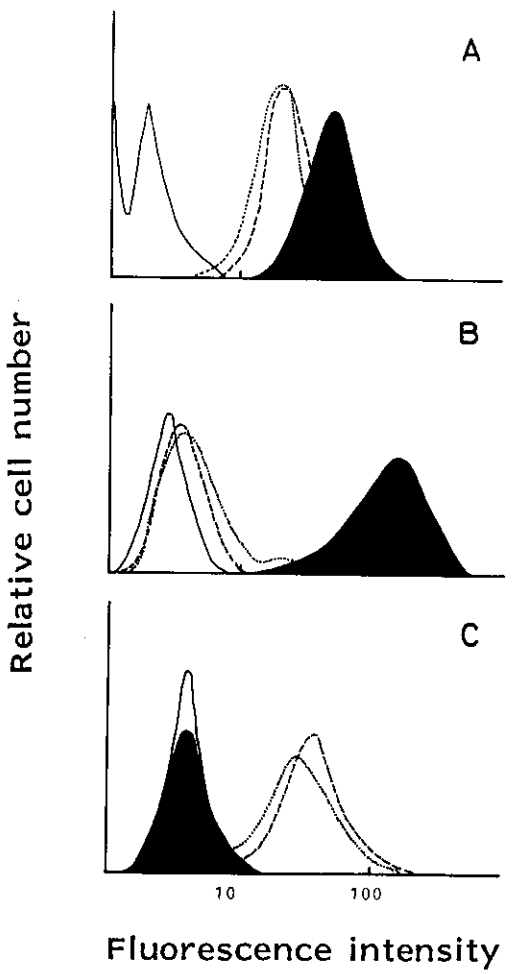

Fig. 1. Differential reactivities of three antibodies with tumor cells. P1.HTR (A), MethA (B) and NS-1 (C) tumor cells were stained by means of the fluorescent antibody technique with anti-P1.HTR serum (■), anti-GIX (⬚) and anti-MuMTV (⬚) antisera. Different reactivity patterns with these three antibodies were found on tested tumor cells. Normal mouse serum control is presented at the left end of each picture (□).

RESULTS

Specificity of the anti-P1.HTR serum Tumor cell lines were stained by the fluorescent antibody technique with the anti-P1.HTR serum. As shown in Table I, not only the P1.HTR mastocytoma but also P815 mastocytoma from which P1.HTR had been derived, MethA fibrosarcoma, P388 and L5178Y lymphomas, L1210 B cell-like leukemia, and EL-4, BW5147 and Yac-1 thymomas strongly expressed the antigen reactive with the antiserum on their surface. Very weak staining was observed on W231 B cell lymphoma or NS-1 myeloma. The *in vivo*-maintained P1.HTR and MethA also carried the antigen reactive with the antiserum, ruling out the possibility that the antibody activity demonstrated was primarily directed to some serum component in the culture medium.

To investigate whether some normal tissue antigens were involved in the reaction with the anti-P1.HTR serum, normal mouse thymocytes, bone marrow cells and splenocytes, and fetal liver cells were tested for the reactivity. In contrast to the broad reactivity with the tumor cell lines, this antibody did not react distinctly with any normal tissue cells from either fetal or adult mice.

Table II. Demonstration of G_{IX} and MuMTV Antigens on Various Tumor Cell Lines

Cell line	MFI[a]	
	anti-G_{IX}	anti-MuMTV
P1.HTR	63.1	123.3
P815	44.1	41.1*
MethA	2.5	0.9
L5178Y	62.5	39.5
P388	34.2	9.4*
L1210	85.2	88.5
NS-1	127.6	38.6
BW5147	114.3	38.7
Yac-1	47.2	8.3

a) The means of the MFI values for 2–5 experiments are presented, except for the cases marked *, where the value for one experiment is shown.

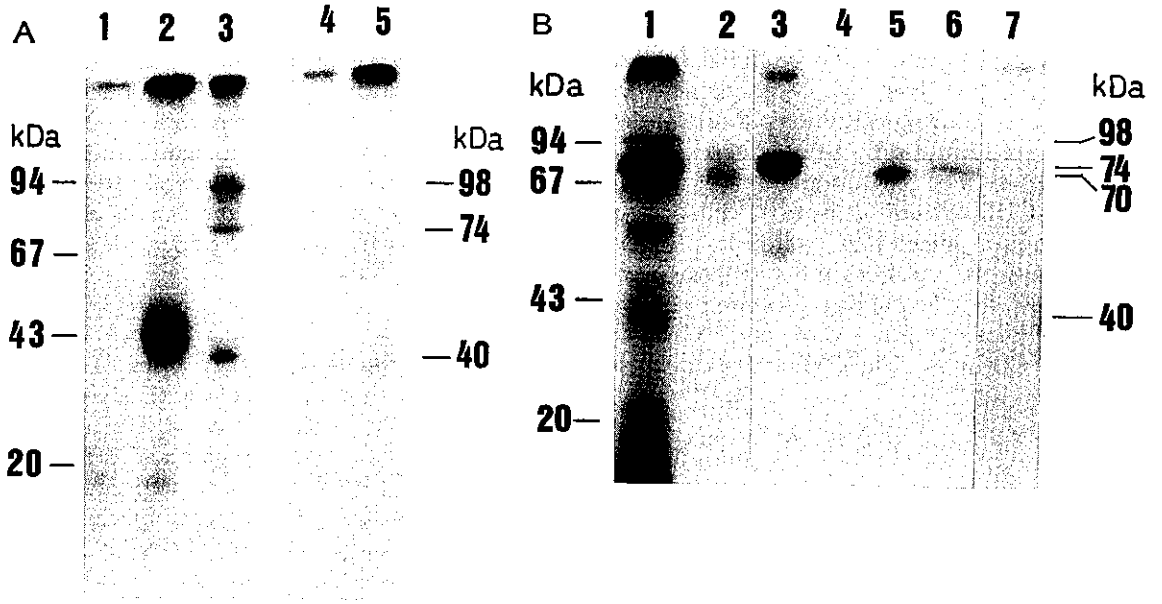

Fig. 2. Identification of cell surface molecules on various cells reactive to the anti-P1.HTR serum. A. Surface ^{125}I-labeled cell lysates from the P1.HTR tumor cells were reacted with normal mouse serum (lane 1), anti-H-2D^d monoclonal antibody (lane 2) and anti-P1.HTR serum (lane 3). The cell lysates from MuMTV-73 tumor cells were reacted with normal mouse serum (lane 4) and anti-P1.HTR serum (lane 5). B. The cell lysates from P1.HTR cells were precipitated with anti-P1.HTR serum (lane 1) and anti-MuMTV antiserum (lane 2); the lysates from MethA cells were precipitated with anti-P1.HTR serum (lane 3) and anti-MuMTV serum (lane 4); the cell lysates from L1210 (lane 5) and NS-1 (lane 6) tumor cells and normal spleen cells (lane 7) were also precipitated with anti-P1.HTR serum.

Characterization of the antigen reactive to the anti-P1.HTR antiserum Further experiments were carried out to clarify the relationship between the antigens reactive to the anti-P1.HTR serum and the known MuLV or MuMTV-associated antigens, which should be carried by various tumor cell lines. The patterns of reactivity of different tumor cells with anti-G_{IX} and anti-MuMTV antisera are shown in Fig. 1 and Table II. P1.HTR, P815, L5178Y, L1210, and BW5147 tumor cells, which reacted with the anti-P1.HTR serum, showed definite reactivity with both anti-G_{IX} and anti-MuMTV antisera. However, the P388 and Yac-1 tumor cells reacted significantly with anti-G_{IX} antibody only; the MethA tumor cells that strongly reacted with the anti-P1.HTR serum did not bind the anti-G_{IX} or anti-MuMTV antibody; and the NS-1 tumor cells that barely reacted with the anti-P1.HTR serum bound significant amounts of the anti-G_{IX} and anti-MuMTV antibodies. These results suggested that the antigens reactive with the anti-P1.HTR antiserum are not identical with any of these virus-associated antigens.

Biochemical identification of the antigens reactive to the anti-P1.HTR antiserum Further effort was made to identify biochemically the cell-surface components of various cell lines reacting with the anti-P1.HTR serum (Fig. 2). As we reported previously, the anti-P1.HTR serum precipitated 40 kDa, 74 kDa and 98 kDa molecules as major antigens from ^{125}I-labeled P1.HTR cells. The 74 kDa molecule was precipitated from all tumor cell lines tested, such as P1.HTR, MethA, L1210, NS-1 and MMTV-73, although the density of precipitation bands varied among different cell lines. The 40 kDa molecule, which was discriminated from the class I MHC (H-2D) molecule, was precipitated in a large amount from P1.HTR and in a small amount from MuMTV-73. In addition, a large amount of an 80 kDa molecule was precipitated from MethA tumor and a small amount of it from P1.HTR. However, the anti-P1.HTR serum precipitated the 98 kDa molecule only from P1.HTR but not from any other cell lines tested. None of those molecules found in tumor cells was precipitated from the normal spleen cells by the antiserum. On the other hand, the anti-MuMTV serum precipitated a 70 kDa molecule, which was discriminated from the 74 kDa molecule, from P1.HTR tumor cells but not from MuMTV-free MethA tumor.

Specificity of anti-P1.HTR CTL It might be that MuLV or MuMTV-related antigens or other serologically cross-reactive antigens worked as the transplantation antigen

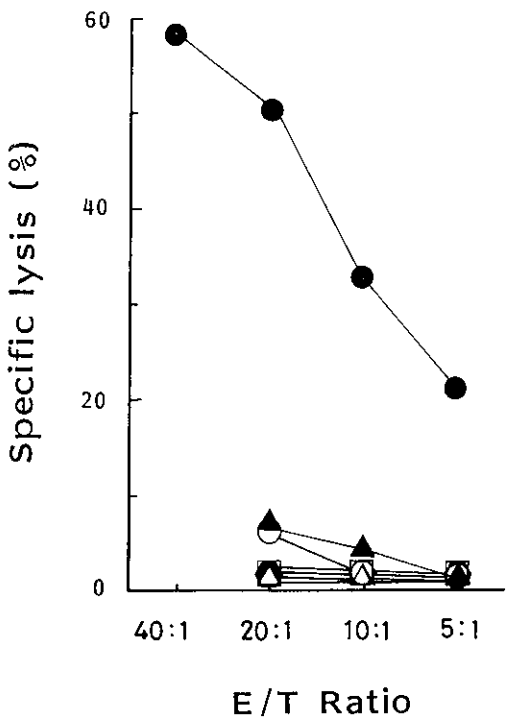

Fig. 3. Demonstration of P1.HTR-specific CTL. CDF_1 mice were inoculated with *H-2-erbB* transfectant first and then challenged 3–5 times with P1.HTR tumor cells. Spleen cells obtained from these mice were sensitized with P1.HTR cells *in vitro* and assayed for the lytic activities against P1.HTR (●), MethA (▲), L5178Y (○), L1210 (□), NS-1 (△), EL-4 (⬤) and BW5147(⬡) tumor cells.

giving rise to cross-reactive protection in our system. We therefore tested whether the tumor cells which serologically reacted with the anti-P1.HTR serum and anti-MuLV or anti-MuMTV antibody could be the target of CTL from the P1.HTR tumor-immunized mice. As shown in Fig. 3, the CTL derived from the mice immunized with the transfectant and P1.HTR tumor cells specifically lysed P1.HTR tumor cells, but not fully syngeneic L1210 or L5178Y tumor cells, H-2 syngeneic MethA or NS-1 tumor cells or allogeneic EL-4 or BW5147 tumor cells. This specificity of CTL immunity corresponded to our previous result on transplantation immunity in mice for rejection of the inoculated tumor.[2] These results suggested that the antigen recognized by the CTL was not identical to any of the serologically cross-reactive tumor antigens or intrinsic retroviral antigens.

DISCUSSION

This study has demonstrated that the tumor-associated antigens on P1.HTR tumor cells which were reactive to the antiserum raised in mice immune to *H-2K-erbB* gene transfectant and parental P1.HTR tumor are rather widely distributed among many established murine tumor cell lines (Table I). The possibility that these antigens are associated with bovine serum components used for cell culture was ruled out because they were detectable on both *in vivo*- and *in vitro*-maintained cell lines. They were absent on either adult or fetal normal tissues and therefore were not any of the major normal tissue differentiation antigens. They consisted of immune-precipitable major 40 kDa, 74 kDa and 98 kDa molecules and were discriminated from MuLV(G_{IX}) or MuMTV antigens that were found on many of the cell lines tested as a 70 kDa molecule (Figs. 1 and 2, Table II). The 74 kDa molecule was precipitated from all of 5 tumor cell lines tested, including the NS-1 tumor that was only weakly reactive to the antiserum, by the fluorescent antibody technique. This suggested that the 74 kDa molecule was primarily responsible for the observed cross-reaction, although the exact nature of this molecule is not known.

On the other hand, the anti-tumor CTL generated in mice immune to the *H-2K-erbB* transfectant and parental P1.HTR tumor specifically lysed P1.HTR cells, but not fully syngeneic L1210 or L5178Y cells or H-2-syngeneic NS-1 or MethA cells, even though many of those tumor cell lines carry MuLV/MuMTV antigens (Fig. 3). The demonstrated MuLV/MuMTV antigens should not therefore be involved in the CTL immunity generated, although the possibility that some microbial antigens other than these demonstrated here are included remains to be ruled out. In any case, the 74 kDa molecule was demonstrated not only in P1.HTR cells but also in L1210, MethA and NS-1 cells, which were not killed by anti-P1.HTR CTL. On the other hand, the 98 kDa molecule and 40 kDa molecule were found preferentially in P1.HTR cells, although the 40 kDa molecule was also detected in MuMTV-73 cells. This finding suggests that the 98 kDa molecule may be involved in the CTL immunity, although we have no direct evidence that this is the case. In our previous study, the anti-P1.HTR serum failed to block the CTL activity against the parental P1.HTR tumor cell. This may not, however, argue against the above-mentioned view since the processed peptide recognized by CTL together with self MHC class I antigen may not be identical to the native unprocessed cell membrane antigen seen by the antibody. The size of this molecule is similar to that of tumor antigens which have been reported to be TATA of MethA (96 kDa)[14, 15] and L1210 (90 kDa)[16] in earlier studies. Recent evidence has shown that a number of heat-shock proteins are produced in tumor cells and the TATA found in MethA as a 96 kDa molecule is one of them.[17] It is therefore possible, though not proven, that the 98 kDa molecule on

P1.HTR cells belongs to the heat-shock protein family, which might be polymorphic among different tumor cells. Further extensive analysis will be necessary to prove or disprove this hypothetical view.

Recently, two tum$^-$ transplantation antigens have been identified from P815 mastocytoma by mutagen treatment.[18, 19] The new antigenic peptides derived from constitutively expressed but mutagenized cellular genes were recognized by specific CTL. These mutant genes encoded 60 kDa and 23.5 kDa proteins, respectively, and these tumor transplantation antigens seem not to be related to any of the antigens identified in our system, at least in size. Isolation and sequence analysis of the molecules reactive with anti-P1.HTR serum are needed to clarify the relationship of these antigens with the reported ones.

So far the finding of concomitant increase in both humoral and cell-mediated anti-tumor immunities is rather exceptional. No antibody response was actually demonstrated in mice that had been immunized with another P815 variant tumor described above.[18-21] High titers of antibody accompanying cell-mediated immunity were previously reported in virus-free animals immunized with virally induced tumors.[1, 6] The present system, in which a contribution of coexisting MuLV or MuMTV to the immunity was ruled out, therefore seems unique as regards the marked increase in both humoral and cell-mediated immunities. In this system, the demonstrated tumor antigens of 40 kDa, 74 kDa and 98 kDa were all immunodominant to the co-existing retroviral antigens for both antibody and CTL response. This immunodominancy is probably due to establishment of stronger immunological tolerance to the vertically transmitted retroviral antigens than to the tumor antigens. This in turn suggests that the immune surveillance mechanism is hardly triggered against the endogenous retroviral antigens under conditions where the immune responses to other tumor antigens can be provoked. In addition, the 98 kDa molecule as a potential TATA was immunodominant to 74 kDa and 40 kDa molecules on the same tumor cells for CTL response. The 98 kDa molecule or some other antibody-undetectable peptide should therefore have the ability to associate efficiently with MHC class I molecule for CTL recognition.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of the Japanese Government and by the Toyota Foundation.

(Received January 5, 1991/Accepted April 23, 1991)

REFERENCES

1) Old, L. J. and Stockert, E. Immunogenetics of cell surface antigens of mouse leukemia. *Ann. Rev. Genet.*, **11**, 127–160 (1977).
2) Old, L. J. Cancer immunology: the search for specificity. G.H.A. Clowes memorial lecture. *Cancer Res.*, **41**, 361–375 (1981).
3) Foley, E. J. Antigenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. *Cancer Res.*, **13**, 835–838 (1953).
4) Prehn, R. and Main, J. Immunity to methylcholanthrene-induced sarcoma. *J. Natl. Cancer Inst.*, **1**, 768–778 (1957).
5) Alexander, P. Foetal antigens in cancer. *Nature*, **235**, 137–140 (1972).
6) Brown, J. P., Klitzman, J. M., Hellstrom, I., Nowinski, R. C. and Hellstrom, K. E. Antibody response of mice to chemically induced tumors. *Proc. Natl. Acad. Sci. USA*, **75**, 955–958 (1978).
7) Ding, L., Isobe, K., Iwamoto, T., Yoshida, T., Nagase, F., Kawashima, K. and Nakashima, I. Cytotoxic T lymphocyte recognition of the H-2-erbB hybrid gene product lacking the complete H-2 domain structure. *Int. Immunol.*, **2**, 91–97 (1990).
8) Ding, L., Isobe, K., Yoshida, T., Kawashima, K. and Nakashima, I. Induction of high grade anti-tumor immunity by use of a recombinant H-2K^b-avian erythroblastosis virus erbB gene transfectant. *Cancer Immunol. Immunother.*, **31**, 115–120 (1990).
9) Stockert, E., Old, L. J. and Boyse, E. A. A cell surface allo-antigen associated with murine leukemia virus; implications regarding chromosomal integration of the viral genome. *J. Exp. Med.*, **133**, 1334–1355 (1978).
10) Takahashi, M., Saga, S., Nagayoshi, S., Imai, M., Tsutsui, Y. and Hoshino, M. Quantitative analysis of mouse mammary tumor virus in milk in two sublines of RIII/AnOk mice with low and high mammary tumor incidence. *Gann*, **74**, 69–76 (1983).
11) Vitetta, E. S., Baur, S. and Uhr, J. W. Cell surface immunoglobulin. II. Isolation and characterization of immunoglobulin from mouse spleen lymphocytes. *J. Exp. Med.*, **134**, 242–246 (1971).
12) Kessler, S. W. Cell membrane antigen isolation with the staphylococcal protein A-antibody adsorbent. *J. Immunol.*, **117**, 1482–1490 (1976).
13) Ando, K., Nakashima, I., Nagase, F., Isobe, K., Kawashima, K., Hasegawa, Y., Yoshida, T., Iwamoto, T., Hasegawa, T., Muro, Y., and Ohashi, M. Induction and characterization of minor histocompatibility antigens-specific primary cytotoxic T lymphocyte responses *in vitro*. *J. Immunol.*, **140**, 723–729 (1988).
14) Srivastava, P. K., Deleo, A. B. and Old, L. J. Tumor

rejection antigens of chemically induced sarcomas of inbred mice. *Proc. Natl. Acad. Sci. USA,* **83,** 3407–3411 (1986).

15) Srivastava, P. and Old, L. J. Individually distinct transplantation antigens of chemically induced mouse tumors. *Immunol. Today,* **9,** 78–83 (1988).
16) Yokochi, T., Kawashima, K., Nakashima, I., Nagase, F., Isobe, K., Nagura, E., Yamada, K., Miyada, T. and Kimura, Y. Identification and characterization of a unique tumor associated surface antigen on L1210 leukemia cells recognized by semisyngeneic antisera. *Cancer Res.,* **47,** 1006–1009 (1987).
17) Kaufmann, S. H. E. Heat shock proteins and the immune response. *Immunol. Today,* **11,** 129–134 (1990).
18) Lurquin, C., Pel, A. V., Mariame, B., Plaen, E. D., Szikora, J., Janssens, C., Reddehase, M. J., Lejeune, J. and Boon, T. Structure of the gene of tum$^-$ transplantation antigen P91A: the mutated exon encodes a peptide recognized with L^d by cytolytic T cells. *Cell,* **58,** 293–303 (1989).
19) Sibille, C., Chomez, P., Wildmann, C., Pel, A. V., Plaen, E. D., Maryanski, J. L., Bergeyck, V. and Boon, T. Structure of the gene of tum$^-$ transplantation antigen P198: a point mutation generates a new antigenic peptide. *J. Exp. Med.,* **172,** 35–45 (1990).
20) Boon, T. Tum$^-$ variants: immunogenic variants obtained by mutagen treatment of tumor cells. *Immunol. Today,* **6,** 307–311 (1985).
21) Wolfel, T. Pel, A. V., Plaen, E. D., Lurquin, C., Maryanski, J. L. and Boon, T. Immunogenic (Tum$^-$) variants obtained by mutagenesis of mouse mastocytoma P815. *Immunogenetics,* **26,** 178–187 (1989).