Separation of the Tumor Rejection Antigen of Rous Sarcoma Virus-induced Murine Fibrosarcoma

Takashi Suda, Jun Shimizu, Yumiko Mizushima, Hiromi Fujiwara[*1] and Toshiyuki Hamaoka
Department of Oncogenesis, Institute for Cancer Research, Osaka University Medical School, 1-1-50, Fukushima, Fukushima-ku, Osaka 553

The tumor antigen capable of inducing tumor resistance (tumor rejection antigen; TRA) was separated and some of its physicochemical properties were characterized. Cytosol and plasma membrane fractions were separated from Rous sarcoma virus (RSV)-induced CSA1M tumor cells. Immunization with membrane but not cytosol fraction of these tumor cells together with complete Freund's adjuvant resulted in complete protection against subsequent challenge with viable CSA1M cells. The TRA activity contained in the membrane fraction was recovered in the sodium dodecyl sulfate (SDS)-solubilized fraction after the SDS-extraction of CSA1M membranes. This CSA1M SDS-solubilized preparation gave protection against syngeneic RSV-induced CSA9F tumor cells as well as the homologous tumor cell type, but failed to induce resistance to RSV-unrelated tumor cells. The membrane or SDS-solubilized fraction from RSV-unrelated tumor cells was unable to generate anti-CSA1M protective immunity. Physicochemical analyses have demonstrated that TRA activity in the SDS-solubilized fraction was completely abolished by treatment with proteinase K but was only marginally affected after treatment with glycosidase mixture. When the SDS-solubilized preparation was applied to a Sephacryl S-300 superfine column, TRA activity was recovered in the range of molecular weight of 50–90 kD. Further fractionation of this TRA-positive fraction by SDS-polyacrylamide gel electrophoresis revealed that the molecular size of TRA is 56–68 kD. These results indicate that membrane proteins which were isolated from CSA1M tumor cells and have a molecular size of about 60 kD are capable of inducing RSV-induced tumor-specific *in vivo* protective immunity.

Key words: Tumor rejection antigen — RSV-induced tumor

It has been established that tumor-associated antigens (TAA)[*2] are expressed on and/or in malignant cells transformed by various types of viruses and that such TAA are common among different tumor cells in which transformation is induced by the identical virus.[1-4] These common TAA in virus-induced tumor cells include cell surface viral structural proteins and transformation-associated non-viral structural proteins,[5,6] although whether the latter type of TAA can function as tumor rejection antigen (TRA) has not been determined.

In the transformation of mammalian cells by Rous sarcoma virus (RSV), the expression of viral oncogene (v-*src*) has a crucial role in the oncogenesis, whereas transformed cells do not produce viral particles or express viral envelop antigens.[3,5,7] In addition, evidence that immunization of mice with RSV particles fails to induce immune resistance against RSV-induced syngeneic tumor cells has indicated that TAA of RSV-induced tumor cells, if any, represent an example of transformation-related non-viral structural proteins.[3] Thus, this model could provide a convenient system for investigating the relationship between virus oncogenesis and the expression of transformation-related TAA as well as the possibility that such TAA function as TRA.

In the present study, we have isolated and partially characterized TRA from RSV-induced tumor cells. The results demonstrate that TRA can be obtained in SDS-solubilized

---

[*1] To whom correspondence should be addressed.
[*2] Abbreviations used in this paper: TAA, tumor-associated antigen; RSV, Rous sarcoma virus; SDS, sodium dodecyl sulfate; TRA, tumor rejection antigen; APMSF, (α-amidinophenyl)methanesulfonyl fluoride; 2-ME, 2-mercaptoethanol; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; CFA, complete Freund's adjuvant; MTD, mean tumor diameter; SAS, saturated ammonium sulfate.

fraction of plasma membranes from RSV-induced CSA1M fibrosarcoma, which had previously been established in BALB/c strain. This SDS-solubilized protein was capable of producing protection against challenge with RSV-induced tumor cells including CSA1M but not with RSV-unrelated syngeneic tumor cells. A series of Sephacryl S-300 gel electrophoresis in the presence of SDS and SDS-polyacrylamide gel electrophoresis (PAGE) has revealed that the TRA has an apparent molecular weight of about 60 kD. The relation of this TRA to molecules which have previously been described to be generated in RSV-induced tumor cells is discussed.

MATERIALS AND METHODS

Mice   Male BALB/c mice were obtained from Shizuoka Experimental Animal Laboratory, Shizuoka, and used at 6–9 weeks of age.

Tumor   RSV-induced CSA1M and CSA9F fibrosarcomas[8] were kindly provided by Dr. T. Yoshida, Hamamatsu Medical College, Hamamatsu. N-Nitroso - N - methylurethane - induced colon 26 adenocarcinoma[9] was developed originally by Dr. T. Yamori, Cancer Chemotherapy Center, Tokyo. These three tumor cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum at 37° in a humidified atmosphere with 5% CO_2. 3-Methylcholanthrene-induced Meth A sarcoma and radiation-induced RL♂1 leukemia were maintained in ascitic form. All tumors were of BALB/c origin.

Preparation and Solubilization of Plasma Membrane Fraction of Tumor Cells   Plasma membrane fractions were prepared according to the method of Tsushima and Friesen[10] with some modifications. Briefly, solid tumors of about 10 mm in diameter were taken out, divided into small pieces and homogenized in 5 volumes of $0.3M$ sucrose solution with a glass homogenizer. This homogenate (first homogenate) was filtered through 2 layers of gauze, and the filtrate was further homogenized with a Polytron PT-10 (Brinkmann) for 1 min, with the dial set at 7. The second homogenate was centrifuged at $1,500g$ for 25 min, and the resulting supernatants were centrifuged sequentially at $15,000g$ for 25 min, and $100,000g$ for 90 min. The $100,000g$ supernatant and pellet were used as the cytosol and plasma membrane fraction, respectively. The $100,000g$ pellet was suspended in $25mM$ Tris-HCl buffer (pH 7.6) containing $10mM$ $CaCl_2$ and 1% aprotinin (Sigma) and was homogenized with a Teflon-glass homogenizer with 10 strokes. Aliquots of this plasma membrane fraction were kept frozen at $-70°$ until use.

The plasma membrane fraction was extracted with 4 volumes of 0.25% SDS in Tris-borate-EDTA (TBE, 10.75 g:5.04 g:0.39 g/liter, pH 8.35) buffer containing $2mM$ (α-amidinophenyl)methanesulfonyl fluoride (APMSF; Wako Pure Chemical Industries) at 37° for 30 min, and the insoluble matrix was removed by centrifugation at $10,000g$ for 30 min. In some experiments, 0.5% sodium deoxycholate, 1% Nonidet P-40 or $30mM$ octyl-β-glucoside was used to solubilize the membrane fraction instead of SDS.

Protein Determinations   Protein concentrations were estimated by the method of Lowry et al.[11] with bovine serum albumin as a standard.

Ammonium Sulfate Precipitation   SDS-solubilized membrane proteins from CSA1M were fractionated by ammonium sulfate precipitation. Saturated ammonium sulfate (SAS) solution was slowly added to the SDS-solubilized fraction (approximately 10 mg protein/ml) to achieve 20% saturation, stirred for 1 hr at room temperature, and centrifuged at $10,000g$ for 20 min. The resulting pellet was dissolved in 0.2% SDS-phosphate buffer saline (PBS) for biological assay or in 0.2% SDS-TBE containing 2% 2-mercaptoethanol (2-ME) and $2mM$ APMSF for further purification, respectively. Insoluble aggregates were removed by centrifugation at $10,000g$ for 20 min.

SDS-Gel Filtration on Sephacryl S-300 Superfine Column   Gel filtration of the 20% SAS fraction in the presence of SDS was performed according to the method of Schreurs et al.[12] except for the utilization of Sephacryl S-300 gel (Pharmacia) instead of Sephacryl S-400 gel. Briefly, 15 mg protein of the 20% SAS precipitate fraction dissolved in 0.2% SDS-TBE containing 2% 2-ME was applied to a column (15×570 mm) equilibrated with 0.2% SDS-TBE buffer and elution was performed at room temperature. The eluate (8 ml/hr) was monitored at 280 nm, and fractions of 4 ml each were collected.

SDS-PAGE   Analytical and preparative SDS-PAGE were carried out by the method of Laemmli.[13] In analytical SDS-PAGE, proteins were stained with Coomassie blue R-250. In preparative SDS-PAGE, 3 to 6 mg protein of the active fraction from gel filtration was applied to a 7 $\times 120 \times 110$ mm 10% polyacrylamide slab gel and electrophoresis was performed at 4° at 100 mA for 6 hr. A slab gel was sliced into 5 mm sections, and each slice was enclosed in a dialysis bag with 5 ml of electrode buffer (identical to the electrode buffer for SDS-PAGE). The bags were immersed in a shallow layer of electrode buffer in an electrophoresis tank and proteins were electroeluted. Electroelution was performed in a cold chamber (4°) at 25 V overnight and the polarity of the electric current was reversed for the last 30 min.

Enzyme Treatments SDS-solubilized fraction and untreated CSA1M membrane fraction were treated with 200 μg (1 unit) of proteinase K (Sigma) for 3 hr at 37° and 100 μg of glycosidase mixture from *Turbo cornutus* (Seikagaku Kogyo) overnight at 37°, respectively.

In vivo **Tumor Rejection Assay** Male BALB/c mice (4/group) were immunized subcutaneously at the base of the tail with 50 μl of test sample (membrane fraction or SDS-solubilized fraction) emulsified with an equal volume of complete Freund's adjuvant (CFA). Two weeks later, all mice were challenged intradermally in the right flank with 2×10^5 CSA1M tissue culture cells or appropriate numbers of various tumor cells. Growth was monitored from day 9 to day 41 after the tumor inoculation. The tumor diameter of each tumor was determined from the average of two diameters at right angles to each other and the tumor growth of the group was expressed as the mean tumor diameter (MTD)±SE of 4 mice/group. Percent growth inhibition of the tumors was calculated as follows: % growth inhibition = (1 − MTD in experimental group/MTD in control group) × 100.

RESULTS

Cellular Localization of CSA1M Tumor Rejection Antigen Male BALB/c mice were inoculated intradermally (id) with 10^6 viable CSA1M tumor cells followed by the surgical resection of the tumor. These mice produced complete protection against challenge with 2×10^5 viable CSA1M tumor cells, indicating the existence of TRA on and/or in CSA1M

tumor cells (Table I, Group 2). To investigate the cellular localization of the molecule(s) responsible for the above tumor resistance, cytosol and plasma membrane fractions were separated from CSA1M tumor cells. Male BALB/c mice were immunized with either of these subcellular fractions emulsified in CFA, and were challenged with CSA1M cells 2 weeks later. The results of Table I also show that sensitization with cytosol fraction failed to induce complete protection against the

Table I. Induction of Anti-CSA1M Protective Immunity by Immunization with CSA1M Cells or Cell Membrane Fraction

Group	Immunized with	Dose (μg)	Tumor growth[c]
1	None		13.0±0.6
2	Cells[a]		<2.0
3	Cytosol[b]	36	12.7±0.6
4		360	7.8±3.9
5	Cell membrane[b]	36	6.8±3.4
6		180	<2.0
7		360	<2.0

a) BALB/c male mice were inoculated with 10^6 CSA1M cells followed by surgical resection of the tumor mass two weeks later.
b) Mice were immunized with various doses of fractions in CFA emulsion. The protein dose in each fraction was determined by the method of Lowry.
c) Tumor growth was expressed as the mean tumor diameter in mm±SE of 4 mice/group on day 41.

Table II. Induction of Anti-CSA1M Protective Immunity by Immunization with Detergent-solubilized Fractions

Exp.	Group	Immunizing protein		Tumor growth[a]	% growth inhibition
		Solubilized with	Dose (μg)		
I	1	SDS	0[b]	15.0±0.3	—
	2	SDS	36	10.3±1.0	31
	3	SDS	100	4.8±3.2	68
	4	SDS	180	<2.0	100
II	1	—	0[c]	14.6±1.8	—
	2	SDS	180	<2.0	100
	3	Sodium deoxycholate	180	<2.0	100
	4	Nonidet P-40	180	<2.0	100
	5	Octyl-β-glucoside	180	<2.0	100

a) Tumor growth was determined on day 41.
b) Mice were immunized with CFA emulsion containing approximately the same amount of SDS without membrane protein.
c) Mice were immunized with CFA alone.

CSA1M challenge although weak but detectable tumor growth inhibition was obtained by administration of a high dose (360 μg/mouse) of cytosol fraction (Group 4). In contrast, immunization with membrane fraction resulted in complete protection when the immunizing dose was 180 μg/mouse or more (Groups 6 and 7).

Since anti-CSA1M TRA activity was found predominantly in the plasma membrane fraction rather than in the cytosol fraction of CSA1M cells, solubilization of the membrane fraction to obtain TRA molecules was performed with the use of various types (ionic or non-ionic) of detergents. The results of Table II demonstrate that when the plasma membrane fraction was extracted with 0.2% SDS, an ionic detergent, anti-CSA1M activity was successfully recovered in the SDS-solubilized fraction. It should be noted that tumor growth was not affected by preimmunization with CFA emulsion containing 0.2% SDS solution without membrane protein as a control (Group 1 in Exp. I). It was also shown that another ionic detergent (0.5% sodium deoxycholate) and non-ionic detergents (1% Nonidet P-40 or 30mM octyl-β-glucoside) were all capable of solubilizing the CSA1M TRA activity. However, the solubilization of membranes with non-ionic detergents such as Nonidet P-40 or octyl-β-glucoside resulted in appreciably lower recovery of TRA activity (TRA activity based on the protein concentration was almost comparable in fractions solubilized with various detergents but the protein recovery by non-ionic detergent was approximately 20% of that by ionic detergent). Therefore, SDS, a typical ionic detergent, was utilized for the solubilization throughout the present study.

Specificity of TRA Recovered in SDS-solubilized Fraction In order to determine whether TRA recovered in the SDS-solubilized fraction from CSA1M membranes possesses tumor specificity, BALB/c male mice preimmunized with the SDS-solubilized membrane molecules were challenged with various syngeneic tumor cells. As shown in Table III, the immunization with CSA1M SDS-solubilized molecules resulted in complete protection against challenge with RSV-induced CSA9F as well as CSA1M cells. However, the same immunization failed to

induce any significant inhibiting effect on the growth of RSV-unrelated Meth A sarcoma, colon 26 adenocarcinoma or radiation-induced RL$1 leukemia cells. Conversely, growth inhibition of the challenging CSA1M cells was obtained by preimmunizing the membrane fraction from RSV-induced CSA1M or CSA9F but not from RSV-unrelated tumor cells (Exp. I of Table IV).

Table III. Specificity of Tumor Rejection Immunity Induced by SDS-solubilized CSA1M Membrane Fraction

Challenge[a] with	Tumor growth in mice[b]		% growth inhibition
	Normal	CSA1M-immune[c]	
CSA1M	7.8±0.3	<2.0	100
CSA9F	10.4±0.4	<2.0	100
Meth A	28.0±1.1	27.1±0.8	3
Colon 26	12.8±0.7	13.1±1.5	0
RL$1	19.1±1.7	22.4±1.3	0

a) Mice were challenged with 2×10^5 CSA1M cells, 10^5 CSA9F cells, 2×10^5 Meth A cells, 2.5×10^4 colon 26 cells or 10^5 RL$1 cells.
b) Tumor growth was determined on day 25.
c) Mice were immunized with SDS-solubilized CSA1M membrane fraction (200 μg protein).

Table IV. Capability of Membrane Fractions or SDS-solubilized Membrane Fractions from Various Tumor Cell Lines to Induce Anti-CSA1M Protection

Exp.	Immunized with[a]	Growth of CSA1M tumor[b]	% growth inhibition
I	none	9.6±1.1	—
	CSA1M	<2.0	100
	CSA9F	3.6±2.1	63
	Meth A	11.1±0.7	0
	Colon 26	8.5±2.9	11
II	none	11.8±0.9	—
	CSA1M	2.2±2.2	81
	Meth A	10.8±1.3	8

a) Mice were immunized with 400 μg of membrane fraction (Exp. I) or 200 μg of SDS-solubilized membrane fraction (Exp. II) from each tumor cell line 2 weeks before challenge with 2×10^5 viable CSA1M tumor cells.
b) Tumor growth was determined at day 41.

Table V. Susceptibility of CSA1M TRA Activity to Enzyme Treatments

Exp.	Immunization with SDS-solubilized fraction		CSA1M tumor growth	% growth inhibition
	Treatment	Antigen dose (μg)		
I	—	0	13.4±1.0	—
	—	165	<2.0	100
	proteinase K[a]	165	12.9±0.4	4
II	—	0	13.0±0.7	—
	—	100	2.8±2.8	78
	glycosidase mixture[b]	100	5.8±3.4	55

a) 1.6 mg protein of SDS-solubilized CSA1M membrane fraction was treated with 200 μg (1 unit) of proteinase K at 37° for 3 hr.

b) 1.6 mg of CSA1M membrane fraction was treated with 100 μg of glycosidase mixture (*Turbo cornutus*) in citrate buffer (pH 5.0) at 37° for 20 hr. After the treatment, the membrane fraction was solubilized with 0.2% SDS.

This was also the case when the growth inhibition of CSA1M cells was tested with the use of SDS-solubilized molecules from CSA1M or Meth A (Exp. II of Table IV). These results indicate the specificity of tumor rejection immunity which was induced by TRA recovered in SDS-solubilized membrane molecules.

Susceptibility of CSA1M TRA Activity to Enzyme Treatment The SDS-solubilized membrane fraction of CSA1M cells was treated with proteinase K. Mice were immunized with the enzyme-treated SDS-solubilized fraction in CFA emulsions. The results of Table V show that the treatment of CSA1 M TRA with proteinase K results in almost complete abrogation of TRA activity. The effect of treatment with glycosidase mixture on TRA activity was examined by using
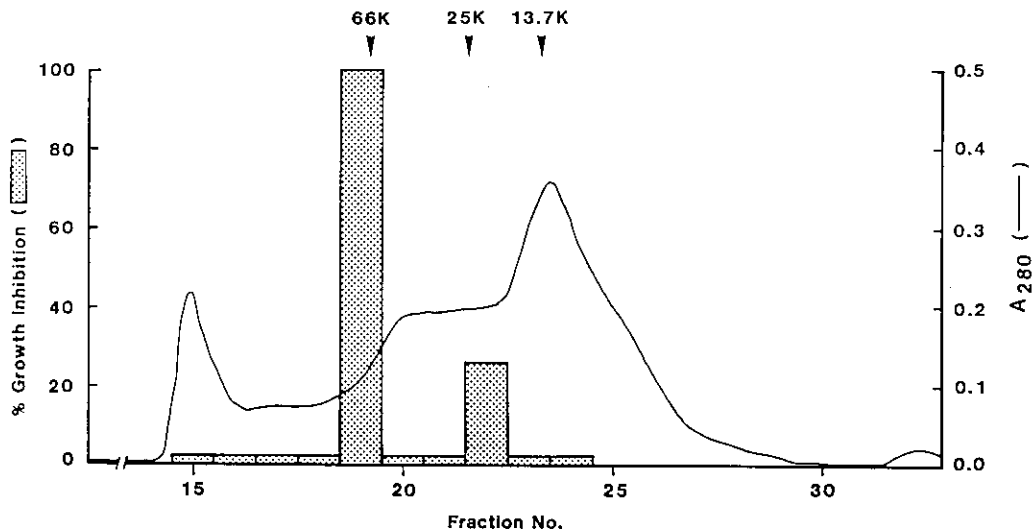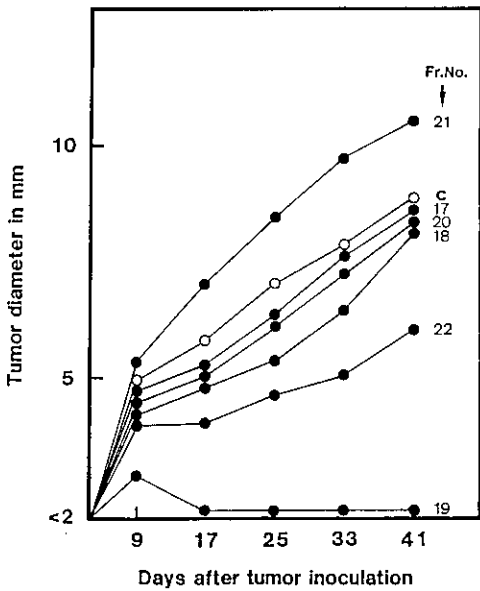

Fig. 1. SDS-gel filtration of SDS-solubilized fraction on a Sephacryl S-300 superfine column. The 20% saturated ammonium sulfate fraction containing 2% 2-ME was fractionated on a Sephacryl S-300 superfine column equilibrated with 0.2% SDS-Tris-borate-EDTA buffer. Elution profile and percent growth inhibition (see footnote of Table II) are indicated by a solid line and dotted bars, respectively. Arrowheads indicate the elution position of molecular weight marker proteins: bovine serum albumin (66 kD), chymotrypsinogen (25 kD) and ribonuclease A (13.7 kD).

Fig. 2. Growth of CSA1M tumor in BALB/c mice immunized with the fractions from SDS-gel filtration. BALB/c mice (4 mice per group) immunized with 50 μl per mice of each fraction (●) or control buffer (○) in CFA emulsion were challenged with 2×10^5 CSA1M cells 2 weeks later.

CSA1M membrane fraction before SDS-solubilization. After the glycosidase treatment, the membrane fraction was solubilized by SDS. Immunization with more than 400 μg of solubilized glycosidase-treated membrane fraction induced complete anti-CSA1M protection as effectively as the untreated solubilized sample at the same dose (data not shown). In order to investigate more accurately the susceptibility to this enzyme, mice were immunized with a dose (100 μg) of solubilized antigen which was capable of reproducibly eliciting 60–80% growth inhibition (see Fig. 4). The results (Table V) also
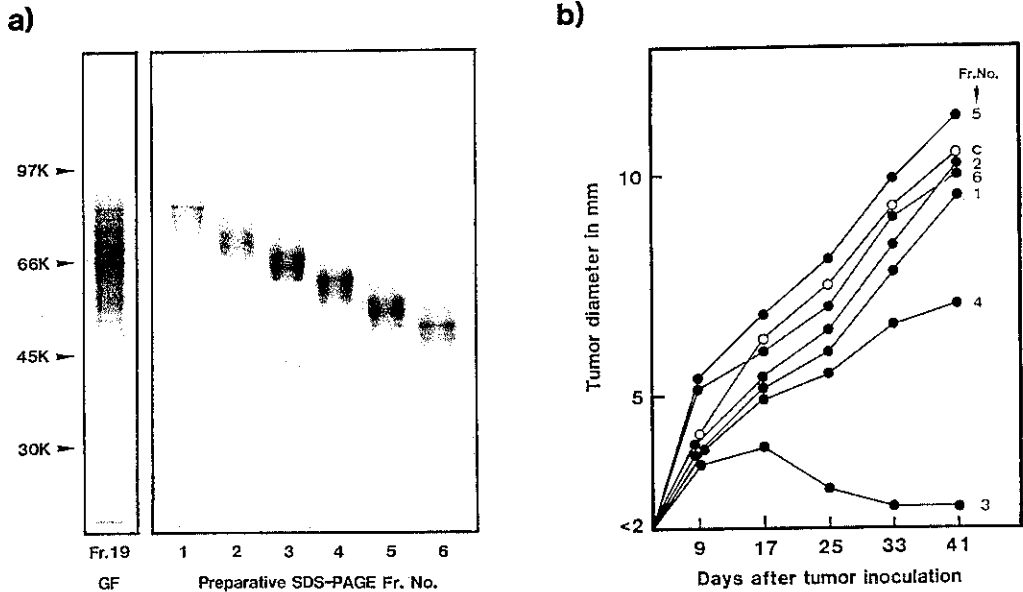

Fig. 3. Purification of TRA activity by preparative SDS-PAGE. a) Analytical SDS-PAGE profile of fractions from preparative SDS-PAGE of Fr. 19 from SDS-gel filtration. Fraction 19 from SDS-gel filtration (Fr. 19 GF) which contains proteins having a molecular size in the range of 50–90 kD (left column) was fractionated by preparative SDS-PAGE. The molecular weight markers were phosphorylase b (97 kD), bovine serum albumin (66 kD), ovalbumin (45 kD) and carbonic anhydrase (30 kD). Electrophoresis was performed under reducing conditions. b) Growth of CSA1M tumor in BALB/c mice immunized with the fractions from preparative SDS-PAGE. BALB/c mice were immunized with 20 μg of each fraction (●) or control buffer (○) in CFA emulsion.

SEPARATION OF TUMOR REJECTION ANTIGEN

demonstrate only a marginal inhibitory effect of the treatment with glycosidase mixture on the activity of CSA1M TRA.

Partial Purification of CSA1M TRA SDS-solubilized CSA1M membrane proteins were fractionated with 20% SAS. Approximately 50% of protein and almost all the TRA activity of SDS-solubilized fraction were recovered in the 20% SAS precipitate. The precipitate was dissolved in buffer containing 0.2% SDS and 2% 2-ME. 2-ME was included in the buffer, since this improved the resolution of gel filtration. The SAS precipitate was applied to a Sephacryl S-300 superfine column equilibrated with 0.2% SDS-TBE buffer. Figure 1 shows a representative elution profile of this column chromatography. Mice were immunized with 50 μl of each fraction and 2 weeks later challenged with CSA1M tumor cells. The ability of each fraction from SDS-gel filtration to inhibit the growth of CSA1M tumor cells is shown in Fig. 2. The percent growth inhibition by each fraction is also shown in Fig. 1. The results clearly demonstrate that complete protection was obtained by immunization with a single fraction (Fraction 19). Although some TRA activity was recovered in Fraction 22, such activity was marginal. These results indicate that the ma-

jority of TRA activity was recovered in Fraction 19. Fraction 19 contained proteins in the molecular weight range of 50–90 kD (the left column of Fig. 3a).

Fraction 19 from gel filtrations was further fractionated by preparative SDS-polyacrylamide gel electrophoresis (PAGE). A sample of Fraction 19 was applied to a $7 \times 120 \times 110$ mm polyacrylamide slab gel and electrophoresis was performed. The gel was then sliced into 5 mm sections, and proteins in each slice were electroeluted. Fractions from preparative SDS-PAGE were analyzed on 0.1% SDS-10% PAGE (Fig. 3a). Thus, Fraction 19 of Sephacryl S-300 gel filtration was divided into 6 fractions. Mice were immunized with 20 μg of each fraction and challenged with 2×10^5 CSA1M cells. As shown in Fig. 3b, TRA activity capable of inducing complete tumor protection was recovered in Fraction 3 containing proteins which have an apparent molecular weight range of 56–68 kD.

A comparison was made of the protein doses of SDS-solubilized fraction (crude preparation) and semi-purified fraction (Fraction 3 of SDS-PAGE) required for inducing 50% inhibition of CSA1M tumor growth (Fig. 4). The slope of the dose-response curve obtained with the semi-purified sample was similar to
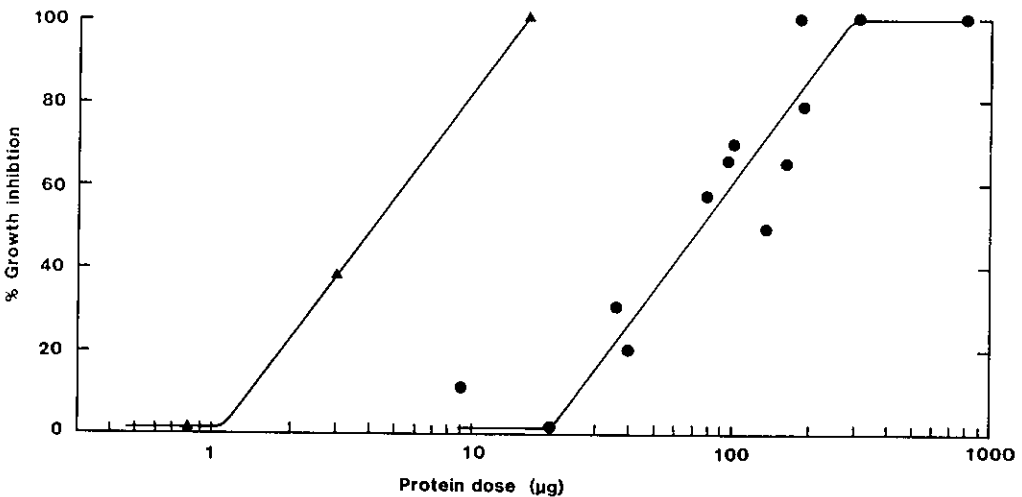

Fig. 4.   Dose-response curves for the immunogenicity of crude and semipurified CSA1M solubilized antigens. Male BALB/c mice were immunized with various doses of crude (SDS-solubilized fraction, ●) and semi-purified fraction (Fr. 3 from preparative SDS-PAGE, ▲) 2 weeks before challenge with 2×10^5 CSA1M cells.

79(3)   1988

that obtained with the crude SDS-solubilized preparation, and the increase in specific TRA activity after preparative SDS-PAGE was approximately 18-fold.

DISCUSSION

The present study demonstrated that a) TRA is localized in the plasma membrane fraction of RSV-induced fibrosarcoma, CSA1M; b) CSA1M TRA activity is solubilized from the membrane fraction with the use of SDS; c) the solubilized TRA functions as a common antigen capable of producing protection against RSV-induced, but not RSV-unrelated, tumor cells; d) this TRA molecule(s) is of protein nature, having an apparent molecular weight of approximately 60 kD.

The results obtained in this study have several implications. First, the fact that CSA1M TRA activity is retained through the process of solubilization by an ionic detergent such as SDS in the presence of 2-ME, which may affect disulfide bond(s), suggests that the fine conformational structure of TRA molecules is not necessarily required for the expression of TRA activity. The relative stability of TRA was also reported by others. Law et al. indicated that the immunogenicity is maintained through the purification process of TRA using SDS in chemically induced Meth A [4] and murine leukemia virus (MuLV)-induced RBL-5 tumor models. [5] The stability of TRA to heat treatment was also reported by Sato et al. in the chemically induced C-C26 tumor model. [6] Thus, it appears that the immunogenicity of TRA is relatively stable to physicochemical treatments in several tumor models.

Second, the TRA obtained from RSV-induced CSA1M tumor cells (BALB/c origin) induced protection against another RSV-induced tumor derived from BALB/c mouse, but failed to produce protection against challenge with tumor cells which were derived from BALB/c strain but not induced by RSV. Conversely, TRA activity capable of inducing anti-CSA1M protective immunity was successfully prepared from two (CSA1M and CSA9F) RSV-induced tumors, but not from RSV-unrelated tumor cells. Moreover, our recent study has revealed that SDS-solubilized antigens prepared from various RSV-induced tumors bearing different H-2 specificities were also capable of producing immune protection against challenge with CSA1M tumor cells in BALB/c mice (to be published). Taken collectively, these observations suggest that the preparation solubilized from CSA1M tumor cell membranes represents TRA which is shared among various RSV-induced tumor cells derived from different strains but is not cross-reactive to RSV-unrelated tumor cells.

Third, the most important and interesting aspect of the TRA in the present study is its relation to molecules which have already been described to be generated in RSV-induced tumor cells. Kotler et al. demonstrated enhanced expression of endogenous MuLV in RSV-induced tumor cells. [17] It was also reported that gp70, the env gene product of endogenous MuLV is a tumor surface antigen of chemically induced or virus-induced tumor cells which is recognized by conventional or monoclonal antibodies. [18, 19] The gp70 is also expressed on CSA1M tumor cell surfaces (unpublished observation). However, the gp70 in SDS-solubilized fraction from CSA1M membranes exhibited an apparent molecular weight of 80 kD when determined by using goat anti-gp70 antibody, and anti-gp70 reactivity was not detected in Fraction 3 from preparative SDS-PAGE, containing CSA1M TRA activity (unpublished observation). These observations make it unlikely that the CSA1M TRA is related to gp70 on these tumor cells.

The molecular weight of CSA1M TRA estimated by SDS-PAGE corresponds with the molecular weight of v-src gene product (pp60$^{\text{v-src}}$). In addition, both of these share the common features that they exist in association with the membrane fraction and successful solubilization requires a potent ionic detergent such as SDS. [20] It is well known that pp60$^{\text{src}}$ is expressed inside plasma membrane as a protein bearing tyrosine kinase activity. However, it was also reported that pp60$^{\text{src}}$ is expressed on cell surfaces of chicken embryonic fibroblasts transformed by Schmidt-Rupin RSV. [21] Therefore, there still exists a possibility that pp60$^{\text{v-src}}$ functions as a cell surface TRA. Alternatively, it could be that transformation of cells by RSV results in the expression of a neoantigen which has a molecular weight of 60 kD but is not related to

pp60^{src}. Phillips *et al.* reported a newly generated tumor cell surface antigen on RSV-induced rat tumor cell surfaces which had an apparent molecular weight of 60 kD, but did not react with the antibody against pp60^{src}.[22] If this is also the case for the TRA of CSA1M tumor cells, the possibility arises that the TRA of CSA1M is a virus-induced transformation-related antigen rather than a viral structural protein or pp60^{v-src}.

Thus, further biochemical characterization of CSA1M TRA could contribute to a better understanding of whether CSA1M TRA is generated as a direct product translated from the oncogene (pp60^{v-src}) or as a product indirectly associated with the oncogenesis. This again emphasizes the advantage of this tumor model for elucidation of the relationship between the expression of TRA and the molecular mechanism of oncogenesis.

REFERENCES

1) Habel, K. Resistance of polyoma virus immune animals to transplanted polyoma tumors. *Proc. Soc. Exp. Biol. Med.*, **106**, 722–725 (1961).

2) Sjögren, H. O., Hellström, I. and Klein , G. Resistance of polyoma virus immunized mice to transplantation of established polyoma tumors. *Exp. Cell. Res.*, **23**, 204–208 (1961).

3) Jonsson, N. and Sjögren, H. O. Further studies on specific transplantation antigens in Rous sarcoma of mice. *J. Exp. Med.*, **122**, 403–421 (1965).

4) Law, L. W., Rogers, M. J. and Appella, E. Tumor antigens on neoplasms induced by chemical carcinogens and by DNA- and RNA-containing viruses: properties of the solubilized antigens. *Adv. Cancer Res.*, **32**, 201–235 (1980).

5) Kurth, R. and Bauer, H. Avian RNA tumor viruses. A model for studying tumor associated cell surface alterations. *Biochim. Biophys. Acta*, **417**, 1–23 (1975).

6) Plata, F., Langlade-Demoyen, P., Abastado, J. P., Berbar, T. and Kourilsky, P. Retrovirus antigens recognized by cytolytic T lymphocytes activate tumor rejection *in vivo. Cell*, **48**, 231–240 (1987).

7) Bauer, H., Bubenek, J., Graf, T. and Allgaier, C. Induction of transplantation resistance to Rous sarcoma isograft by avian leukosis virus. *Virology*, **39**, 482–490 (1969).

8) Yoshida, T. O., Haraguchi, S., Miyamoto, H. and Matsuo, T. Recognition of RSV-induced tumor cells in syngeneic mice and semisyngenic reciprocal hybrid mice. *Gann Monogr. Cancer Res.*, **23**, 201–212 (1979).

9) Yamori, T., Tsuruo, T., Naganuma, K., Tsukagoshi, S. and Sakurai, Y. Isolation and characterization of highly and rarely metastatic clones from murine colon adenocarcinoma 26. *Invasion Metastasis*, **4**, 84–97 (1984).

10) Tsushima, T. and Friesen, H. G. Radioreceptor assay for growth hormone. *J. Clin. Endoclinol. Metab.*, **37**, 334–337 (1973).

11) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275 (1951).

12) Schreurs, V. V. A. M., Boekholt, H. A. and Koopmanschap, R. E. Gel filtration in sodium dodecyl sulphate of hydrophobic muscle proteins on Sephacryl S-400 superfine. *J. Chromatogr.*, **254**, 203–210 (1983).

13) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).

14) DuBois, G. C., Law, L. W. and Appella, E. Purification and biochemical properties of tumor-associated transplantation antigens from methylcholanthrene-induced murine sarcomas. *Proc. Natl. Acad. Sci. USA*, **79**, 7669–7673 (1982).

15) Rogers, M. J., Galetto, G., Hearing, V. J., Siwarski, D. F. and Law, L. W. Purification of a glycoprotein bearing a tumor transplantation antigen specific for Friend, Moloney, and Rauscher MuLV-induced tumors. *J. Immunol.*, **132**, 3211–3217 (1984).

16) Sato, N., Yagihashi, A., Okubo, M., Torigoe, T., Takahashi, S., Sato, T. and Kikuchi, K. Characterization of tumor rejection antigen molecules of chemically induced murine colon tumor C-C26. *Cancer Res.*, **47**, 3147–3151 (1987).

17) Kotler, M., Lerer, I., Moyal, Z. B. and Spira, G. Simultaneous production of mouse endogenous virus and Rous sarcoma virus by Schmidt-Ruppin virus infected mouse cells.

J. Gen. Virol., **40,** 141–150 (1978).

18) Fougereau, M. and Dausset, J. The antigens of chemically induced tumors. *Proc. Fourth Int. Congr. Immunol.,* 659–667 (1980).

19) Nakayama, E., Uenaka, A., DeLeo, A. B., Stockert, E., Obata Y., Ueda, R. and Inui, Y. Molecular basis of an unique tumor antigen of radiation leukemia virus-induced leukemia B6RV2: its relation to MuLV gp70 of xenotropic class. *J. Immunol.,* **136,** 3502–3507 (1986).

20) Hamaguchi, M. and Hanafusa, H. Association of p60[src] with Triton X-100-resistant cel-lular structure correlates with morphological transformation. *Proc. Natl. Acad. Sci. USA,* **84,** 2312–2316 (1987).

21) Barnekow, A., Boschek, B. C., Ziemiecki, A. and Bauer, H. Detection of the src-gene product pp60[src] and its associated protein kinase of the surface of Rous-asrcoma-virus-transformed cells. *Biochem. Soc. Trans.,* **8,** 735–736 (1980).

22) Phillips, E. R. and Ruch, R. J. A nonviral, virus strain-specific antigen expressed on rat cells transformed by avian sarcoma virus. *Cancer Res.,* **46,** 5864–5868 (1986).