Jpn. J. Cancer Res. 80, 879–886, September 1989

The Tumor Rejection Antigen Separated from Rous Sarcoma Virus-induced Murine Fibrosarcoma Exhibits a Molecular Weight of Approximately 60 kD but Differs from Functional pp60^{src}

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The tumor antigen capable of inducing tumor resistance (tumor rejection antigen; TRA) was obtained in a solubilized form by sodium dodecyl sulfate (SDS) extraction of plasma membrane fraction from Rous sarcoma virus (RSV)-induced CSA1M fibrosarcoma cells (BALB/c origin). Analyses by Sephacryl S-300 gel filtration and SDS-polyacrylamide gel electrophoresis revealed that TRA activity was recovered in the fraction with a molecular weight of approximately 60 kD. Unfractionated crude SDS-solubilized preparation contained gp70 as detected by rabbit anti-gp70 antiserum, whereas such reactivity was lost in the fraction exhibiting the molecular weight of about 60 kD. Since this fraction retained pp60^{src} activity, the relation of TRA to pp60^{src} was further investigated. pp60$^{v\text{-}src}$ was also obtained from the lysate of v-*src*-expressing yeast transformant. Immunization of BALB/c mice with such pp60$^{v\text{-}src}$-containing lysate failed to induce any significant tumor protection. The above 60 kD fraction of CSA1M solubilized antigens was allowed to bind to Sepharose beads coupled with anti-pp60^{src} monoclonal antibody and separated into the bead-bound and bead-unbound fractions. The bead-bound fraction that was recovered from pp60^{src}-binding beads (pp60^{src}-positive fraction) did not exhibit the TRA activity. In contrast, immunization with the fraction depleted of pp60^{src} activity (bead-unbound fraction) resulted in potent tumor protection. These results indicate that the solubilized membranous component(s) of CSA1M with a molecular weight of approximately 60 kD, which is distinct from functional pp60^{src}, functions as the TRA against RSV-induced CSA1M tumor cells.

Key words: Tumor rejection antigen— Rous sarcoma virus — pp60^{src}

Cell transformation by virus induces various types of neoantigens on and/or in malignant transformed cells.[1-4] These neoantigens have been divided into two general classes: (a) cell surface viral structural antigens; and (b) transformation-associated non-viral structural proteins.[5-8] The precise nature of many of the neoantigens remains obscure despite extensive studies.[9-11] In particular, how the latter type of antigens are generated during the transformation, where they are expressed (outside or inside of the cell membrane) and whether they act as the tumor rejection antigen (TRA)[5] have not been determined.

In the above context, a Rous sarcoma virus (RSV)-transformed mammalian tumor system might provide a model suitable for analyzing the transformation-related antigens, since RSV-induced tumor cells neither produce viral particles nor express viral structural antigens.[3, 7, 12] An earlier study from our laboratory has demonstrated that RSV-induced murine fibrosarcoma cells generate tumor-associated antigen (TAA) which has an apparent molecular weight of 60 kD and acts as the TRA.[13] TAA of this molecular weight has also been demonstrated to be expressed on several RSV-induced rat sarcoma cell lines.[14, 15] The similarity of the molecular weight of the TRA (our murine model) or TAA (rat models) to that of the *src* gene product, pp60$^{v\text{-}src}$ requires an investigation of the relationship of the TRA or TAA to pp60$^{v\text{-}src}$. Such a study could lead to the elucidation of the direct or indirect role of viral oncogene (v-*src*) in the expression of TAA or transformation-related neoantigens.

The present study demonstrates that immunization of BALB/c mice with the lysate of v-*src*-expressing yeast transformant or with pp60^{src} obtained from RSV-induced CSA1M tumor cells (BALB/c origin) failed to produce anti-CSA1M tumor protection. In contrast, the fraction which contains protein(s) of about 60 kD but is depleted

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[5] Abbreviations used in this paper: TRA, tumor rejection antigen; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; RSV, Rous sarcoma virus; MTD, mean tumor diameter; CFA, complete Freund's adjuvant; ASV, avian sarcoma virus; TAA, tumor-associated antigen.

of pp60^{src} was capable of exhibiting potent TRA activity. These results indicate that the TRA of CSA1M tumor cells is distinct from the functional pp60^{src} molecule.

MATERIALS AND METHODS

Mice and tumor Male BALB/c mice were obtained from Shizuoka Experimental Animal Laboratory, Shizuoka, and used at 6–9 weeks of age. CSA1M fibrosarcoma which was induced in BALB/c mouse with the Schmidt-Ruppin strain of RSV[16] was utilized.

Yeast transformants expressing v-*src* The v-*src*-expressing yeast transformant, (W303-1A/Yvsrc) which was recently established in Dr. H. Hanafusa's laboratory by transfecting the plasmid YEp51 carrying v-*src* into the parental yeast strain W303-1A[17] and control transformant with vector alone (W303-1A/YEp51) were used. These transformants were grown in synthetic medium containing 2% (w/v) raffinose and 0.67% amino acid-free yeast nitrogen base, 1 mg/ml each of tryptophan and histidine, and 1% (w/v) each of uracil and adenine. For the induction of pp60$^{v\text{-}src}$, the yeasts were grown to 10^7 cells/ml, pelleted, resuspended in synthetic medium containing 2% (w/v) galactose instead of raffinose and incubated in a shaker at 37°C for 2.5 h.[17]

Antibodies Rabbit anti-pp60^{src} antiserum[18] and monoclonal anti-pp60^{src} antibody (mAb327)[19] were utilized. Goat anti-murine leukemia virus gp70 antiserum was a generous gift from Dr. H. Yamamoto (Kohchi Medical College, Kohchi).

Solubilization of plasma membrane fraction of tumor cells and partial purification of TRA by SDS-gel filtration and SDS-PAGE Plasma membrane fractions were prepared according to the method of Tsushima and Friesen[20] with some modifications. Briefly, the solid tumor was divided into small pieces and homogenized in 5 volumes of 0.3 *M* sucrose solution. This homogenate (first homogenate) was filtered through 2 layers of gauze, and the filtrate was further homogenized with a Polytron PT-10 for 1 min. The second homogenate was centrifuged at 1,500*g* for 25 min, and the resulting supernatants were centrifuged sequentially at 15,000*g* for 25 min, and 100,000*g* for 90 min. The 100,000*g* pellet was used as the plasma membrane fraction. The pellet (plasma membrane fraction) was resuspended in 25 m*M* Tris·HCl buffer (pH 7.6) containing 10 m*M* $CaCl_2$ and 1% aprotinin (Sigma) and was stored at −80°C until use. The plasma membrane fraction was solubilized 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 2 m*M* (α-amidinophenyl)-methanesulfonyl fluoride (APMSF; Wako Pure Chemical Industries) at 37°C for 30 min, and the insoluble matrix was removed by centrifugation at 10,000*g* for 30 min.

Saturated ammonium sulfate (SAS) solution was slowly added to the SDS-solubilized membrane fraction (approximately 10 mg protein/ml) to achieve 20% saturation, and the mixture was stirred for 1 h at room temperature, then centrifuged at 10,000*g* for 20 min. The resulting pellet was dissolved in 0.2% SDS-TBE containing 2% 2-mercaptoethanol (2-ME) and 2 m*M* APMSF for further purification. Gel filtration of the 20% SAS fraction in the presence of SDS was performed according to the method of Schreurs *et al.*[21] except for the utilization of Sephacryl S-300 gel (Pharmacia) instead of Sephacryl S-400 gel. Briefly, 15 mg protein of 20% SAS precipitate fraction was applied to the column (15×570 mm) equilibrated with 0.2% SDS-TBE buffer and elution was performed at room temperature.

Preparative SDS-PAGE was carried out by the method of Laemmli.[22] Briefly, 3 to 6 mg protein of active fraction from gel filtration was applied to a 10% polyacrylamide slab gel (7×120×110 mm) and electrophoresis was performed at 4°C at 100 mA for 6 h. The gel was cut into 5 mm slices and proteins were recovered from each slice by electroelution in a cold chamber (4°C) at 25 V overnight. The polarity of the electric current was reversed for the last 30 min.

Immunoprecipitation CSA1M membrane fraction (3–4 mg) was solubilized in 500 μl of RIPA buffer [10 m*M* Tris·HCl (pH 7.5), 0.5% Nonidet P-40, 0.5% deoxycholate, 0.02% SDS, 2 m*M* APMSF, 0.02% NaN_3]. After centrifugation in a Microfuge B (Beckman) for 10 min, the supernatant was incubated with 50 μl of monoclonal anti-pp60^{src} antibody(mAb327)-coupled Sepharose CL-4B beads for 1 h at 4°C. The reaction mixture was centrifuged and the supernatant was obtained as the "bead-unbound" fraction. The precipitate (beads) was washed twice with RIPA buffer, resuspended in 50 μl of SDS-PAGE sample buffer, heated at 100°C for 5 min and then centrifuged. The resulting supernatant was obtained as the "bead-bound" fraction. Aliquots of these supernatants (bead-unbound or bead-bound fraction) were submitted to Western blotting and TRA assay.

Western blotting Test samples were subjected to electrophoresis on 0.1% SDS/10% polyacrylamide gels and transferred to nitrocellulose filters (Bio Rad) overnight at 10 V. The filters were then dipped in a blocking solution [10 m*M* Tris-buffered saline (TBS) pH 7.5, 5% bovine serum albumin, 0.5% gelatin] for 2 h. For the detection of pp60^{src}, the filters were incubated with 10 μCi of ^{125}I-labeled anti-pp60^{src} monoclonal antibody and washed for 10–12 h, and immunoreactive bands were detected by autoradiography.

For the detection of gp70, the filters were incubated with a 1:200 dilution of goat anti-gp70 antiserum for 1 h at room temperature, and allowed to react sequentially with a 1:300 dilution of rabbit anti-goat IgG and a

1:2,000 dilution of goat peroxidase-anti-peroxidase. Following washing in TBS containing 0.05% Tween-20 for 2 h, the filters were submitted to reaction with a 4-chloro-1-naphthol substrate solution.

***In vitro* kinase assay** Viral *src*-specific kinase activity was assayed according to the method of Collett and Erikson[23] with some modifications. Briefly, 100–500 μl of test sample was immunoprecipitated with 5 μl of a 1:5-diluted tumor-bearing rabbit serum containing antibodies against the $pp60^{v\text{-}src}$ for 30 min at room temperature, and then the immune complex was collected by absorption on 25 μl of protein A-Sepharose CL-4B beads. The immunoprecipitates were resuspended in 50 μl of standard kinase assay mixture containing 10 m*M* Tris·HCl (pH 7.5), 5 m*M* $MgCl_2$ and 5 μCi of [γ-^{32}P]ATP (5,000 Ci/mmol) and incubated overnight at 4°C. They were then washed twice with TBS, resuspended in SDS-PAGE sample buffer, and heated at 100°C for 5 min. Following centrifugation, the supernatant was subjected to 0.1% SDS/10% polyacrylamide gel electrophoresis. After electrophoresis, gels were briefly stained with Coomassie brilliant blue and phosphorylated protein bands were detected by autoradiography.

***In vivo* tumor rejection assay** Male BALB/c mice (4/group) were immunized subcutaneously at the base of the tail with 50 μl of each test sample which was 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 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 by the mean tumor diameter (MTD) $\pm$ 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) $\times$ 100.

RESULTS

We have confirmed that the TRA of CSA1M tumor cells exhibits a molecular weight of approximately 60 kD.[13] Since anti-CSA1M TRA activity was demonstrated to exist predominantly in the plasma membrane fraction of CSA1M cells,[13] the membrane fraction was solubilized with 0.2% SDS to obtain TRA molecules. SDS-solubilized CSA1M membrane proteins were applied to a Sephacryl S-300 superfine column, and a fraction containing proteins in the molecular weight range of 50–90 kD (TRA-positive fraction) was further fractionated by preparative SDS-polyacrylamide gel electrophoresis (PAGE). Thus, the TRA-positive fraction of Sephacryl S-300 gel filtration was divided into 6 fractions. BALB/c mice were immunized with 20 μg of each fraction and challenged with 2×10^5 CSA1M cells. Three consecutive experiments were performed. The ability of each fraction to inhibit the growth of CSA1M tumor cells is summarized in Fig. 1 in terms of the percent growth inhibition. The growth curve of CSA1M tumor cells in a representative experiment (Exp. II of three consecutive ones) is also shown in Fig. 2. The results demonstrate that TRA activity was recovered mainly in the fraction containing proteins which have an apparent molecular weight of about 60 kD.

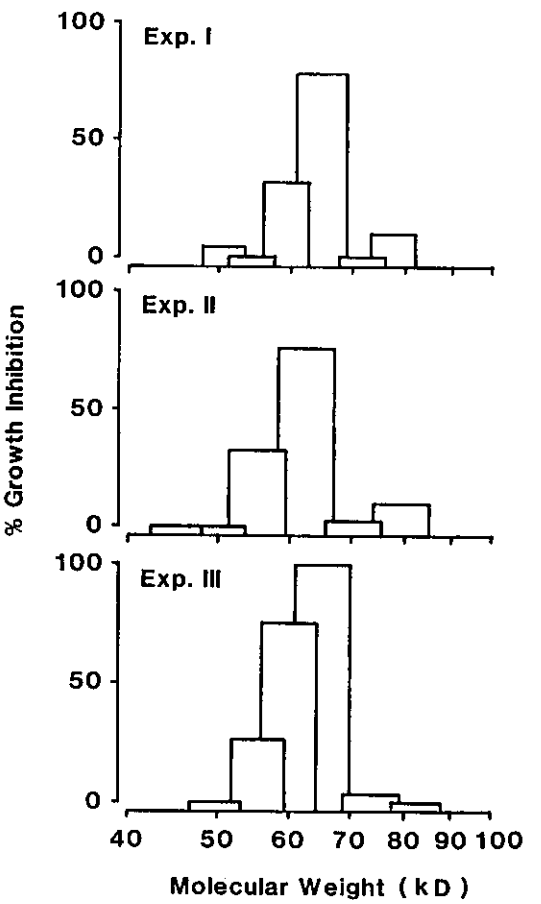

Fig. 1. Separation of TRA activity by preparative SDS-PAGE. The fraction from SDS-gel filtration which contains proteins having a molecular weight in the range of 50–90 kD was fractionated into 6 fractions by preparative SDS-PAGE. BALB/c (4 mice/group) mice were immunized s.c. with each fraction of protein (20 μg) or control buffer emulsified in CFA and challenged with 2×10^5 viable CSA1M tumor cells. Tumor growth was determined on day 41. The capacity of each fraction to generate anti-CSA1M protection was expressed in terms of percent growth inhibition as described in "Materials and Methods."

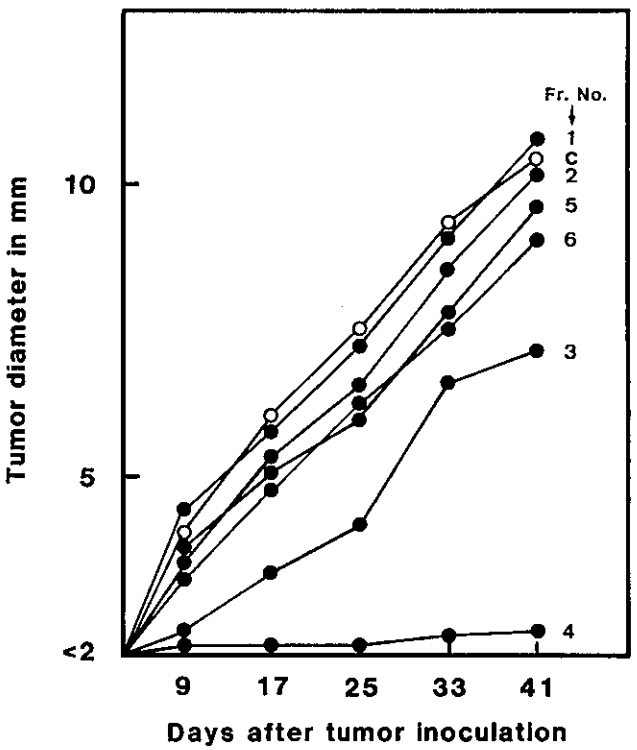

Fig. 2. Growth of CSA1M tumor in BALB/c mice immunized with the fractions from preparative SDS-PAGE. Data represent the mean tumor diameter in mice immunized with buffer (c) or 6 fractions from preparative SDS-PAGE of Exp. II in Fig. 1. Each fraction was numbered from left to right.

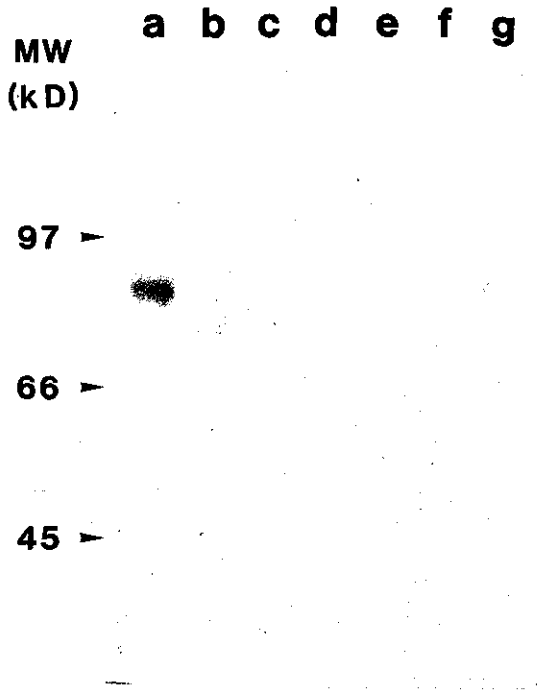

Fig. 3. Western blot with anti-gp70 antibody. Each fraction from preparative SDS-PAGE of CSA1M-solubilized antigens was subjected to electrophoresis on SDS/polyacrylamide gels and transferred to nitrocellulose filters. The filters were incubated with goat anti-gp70 antiserum followed by the sequential reaction with rabbit anti-goat IgG and goat peroxidase-anti-peroxidase. After washing, the filters were submitted to the reaction with a substrate solution. The molecular weight ranges obtained from preparative SDS-PAGE were 76–89 (lane a), 71–82 (lane b), 66–75 (lane c), 60–69 (lane d), 55–62 (lane e) and 50–59 (lane f) kD. Crude CSA1M solubilized proteins were electrophoresed as the initial sample (lane g). The TRA activity was detected in the sample of lane d (data not shown).

With the use of the TRA-positive fraction from SDS-PAGE, we have investigated the relation of the CSA1M TRA to previously described molecules found in murine leukemia virus- or RSV-induced tumor cells. Western blotting analyses using goat anti-gp70 antiserum have revealed that SDS-solubilized crude CSA1M sample contains gp70 having an apparent molecular weight of about 80 kD but this is eliminated in the TRA-positive fraction from SDS-PAGE (Fig. 3). When the existence of pp60^{src} was also examined by using monoclonal anti-pp60^{src} antibody, the pp60^{src} was not detectable in SDS-solubilized crude CSA1M sample, whereas it was found to be enriched in the TRA-positive fraction from SDS-PAGE (Fig. 4). These results raised the possibility that the CSA1M TRA is associated with the existence of pp60^{src}. Since RSV-unrelated colon 26 tumor cells expressing pp60$^{c\text{-}src}$ but not pp60$^{v\text{-}src}$ failed to induce anti-CSA1M tumor protection by cross-immunization,[13] further investigations have focused on analyzing the relationship between the pp60$^{v\text{-}src}$ and TRA.

In order to determine whether pp60$^{v\text{-}src}$ itself is capable of producing tumor protection, the pp60$^{v\text{-}src}$ was obtained from the lysate of the v-*src*-expressing yeast transformant. The existence of tyrosine kinase activity in this preparation was examined by the kinase assay with the use of rabbit anti-pp60$^{v\text{-}src}$ antiserum (Fig. 5). The results illustrate the existence of pp60$^{v\text{-}src}$ in the lysate of yeast transformant as well as crude CSA1M SDS-extract. It should be noted that the tyrosine kinase activity was roughly estimated to be 10–20 times greater in the yeast lysate than in the crude CSA1M SDS-extract on the basis of the amount of applied proteins and the exposure period of autoradiography in the kinase assay. BALB/c mice were immunized with crude CSA1M SDS-extract or the v-*src*-transfected yeast lysate emulsified in CFA and challenged with viable CSA1M tumor cells. The results of Table I demonstrate that administration of CSA1M SDS-extract (200 μg) resulted in complete protection against

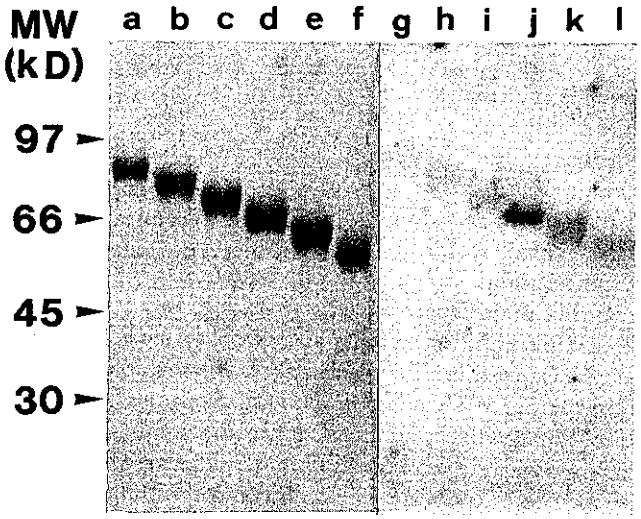

Fig. 4. Western blot with anti-pp60^{src} (327) monoclonal antibody. Portions of the same fractions from SDS-PAGE of CSA1M solubilized antigens as used in Fig. 3 were submitted to electrophoresis on SDS/polyacrylamide gels. The gels were stained with Coomassie brilliant blue (lanes a–f) or transferred to nitrocellulose filters for radioimmunodetection with the use of ^{125}I-labeled anti-pp60^{src} antibody (mAb327)(lanes g–l).

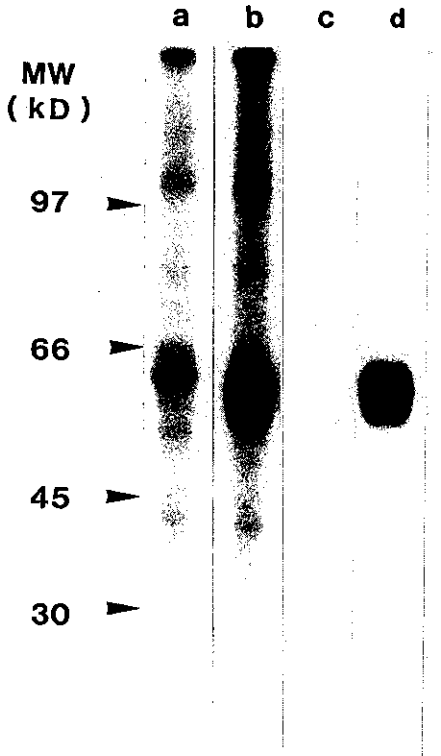

Fig. 5. pp60^{src} kinase activity in the lysate of v-*src*-transfected yeast. CSA1M crude solubilized samples (lanes a and b) or lysates of mock transfectant (lane c) or v-*src*-transfected yeast (lane d) were assayed for *in vitro* kinase activity with the use of control rabbit serum (anti-mouse Ig)(lane a) or tumor-bearing rabbit serum (anti-pp60$^{v\text{-}src}$) (lanes b, c and d).

challenge with CSA1M cells, whereas the yeast lysate containing 10–20 times stronger kinase activity/sample protein weight failed to induce protective immunity.

We additionally investigated whether elimination of pp60^{src} activity from the TRA-positive fraction separated on SDS-PAGE affects the expression of TRA activity. The TRA-positive fraction of SDS-PAGE was mixed with anti-pp60^{src} antibody(mAb327)-coupled Sepharose beads and the bead-bound fraction was separated from the unbound fraction. The unbound fraction was again brought into contact with fresh antibody-coupled Sepharose beads. The first and second bead-bound fractions were recovered by heating in sample buffer for SDS-PAGE. Immunoblotting using monoclonal anti-pp60^{src} antibody capable of reacting with both pp60$^{c\text{-}src}$ and pp60$^{v\text{-}src}$ demonstrates that pp60^{src} was present in the first bead-bound fraction but absent in the unbound and second bead-bound fractions (Fig. 6). These results indicate that almost all pp60^{src} was eliminated from the original TRA-positive sample, yielding pp60^{src}-free "unbound" fraction.

To determine the localization of TRA activity in each fraction, BALB/c mice were immunized with the original TRA-positive fraction from SDS-PAGE, bead-unbound fraction of the first or second bead-bound fraction. The results or Fig. 7 demonstrate that TRA activity comparable to that of the original sample was present in

Table I. Failure of the Lysate from v-*src*-transfected Yeast to Produce Protective Immunity against CSA1M Tumor

Immunization[a] with	Protein dose (μg)	Tumor growth[b] (diameter in mm)	
		Exp. 1	Exp. 2
None	–	10.5±0.9	13.4±1.3
CSA1M crude sample	200	<2.0	<2.0
W303-1A/YEp51 lysate	200	9.8±0.4	11.5±0.5
〃	40	10.4±0.4	ND[c]
〃	8	9.4±1.3	ND
W303-1A/Yvsrc lysate	200	10.8±0.6	12.9±0.8
〃	40	9.8±0.5	ND
〃	8	9.8±0.8	ND

a) BALB/c mice were immunized with crude CSA1M solubilized sample or the lysate from v-*src*-transfected or control yeast at various protein doses as indicated.
b) Tumor growth was determined 41 days after the CSA1M challenge.
c) Not done.

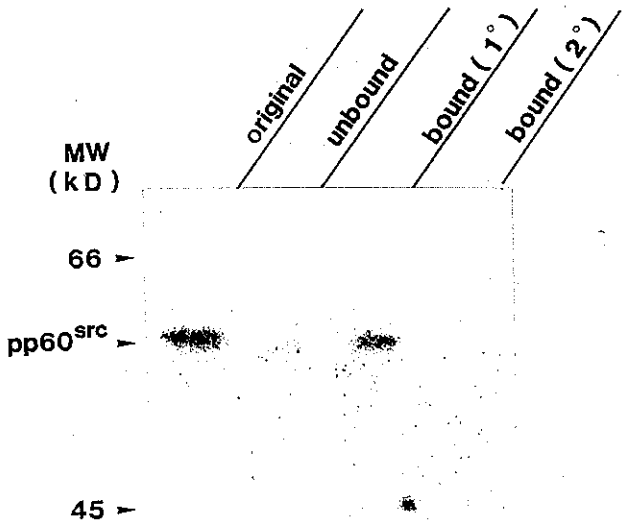

Fig. 6. Localization of pp60^{src} in the partially purified TRA after immunoprecipitation with anti-pp60^{src}-coupled Sepharose 4B. The TRA-positive fraction from SDS-PAGE was immunoprecipitated with anti-pp60^{src} (327) monoclonal antibody-coupled Sepharose 4B and divided into bead-bound or bead-unbound fraction. The bead-unbound fraction was allowed to bind to fresh beads coupled with the 327 antibody, yielding "2nd-bound" fraction. Each fraction was resolved by electrophoresis and proteins were blotted onto nitrocellulose and probed with the ^{125}I-labeled 327 antibody.

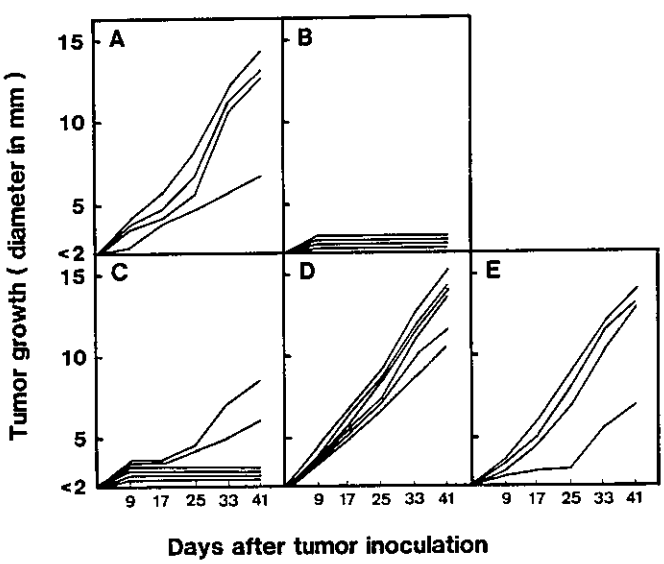

Fig. 7. Growth of CSA1M tumor in BABL/c mice immunized with various fractions obtained in Fig. 6. BALB/c mice were immunized with control buffer (A), TRA-positive fraction from preparative SDS-PAGE (20 μg proteins) (B), 327 antibody-unbound fraction (C), 327-bound (1st) fraction (D) or 327-bound (2nd) fraction (E) emulsified with CFA.

the unbound fraction which had been depleted of pp60^{src}. These results indicate that anti-CSA1M tumor protection does not depend on the existence of serologically active pp60^{src}.

DISCUSSION

Host animals show an immune response to cells transformed by RNA tumor viruses. An element of this response is due to expression of viral structural antigens, but the major part is due to viral transformation-associated antigens. Ample evidence has been presented for the expression of viral transformation-associated neoantigens.[5-11, 24] Most of these antigens are characterized by group specificity with respect to the virus and are, in some cases, shared among different animal species insofar as they are generated by infection with the same virus.[10, 24, 25] The biochemical nature and biological significance of these antigenic molecules have, however, remained to be determined.

Transformation of both avian and mammalian cells by avian sarcoma viruses (ASV) is induced by the virus *src* gene and is invariably accompanied with the appearance of tumor-associated cell surface antigens. Phillips *et al.* have described a tumor surface antigen on several rat sarcoma cell lines induced by the Schmidt-Ruppin strain of ASV.[14, 15] This antigen was immunochemically defined by immunoprecipitation from detergent-solubilized extracts with the use of antiserum from tumor-immunized hosts. We have also isolated the SDS-solubilized tumor-associated antigens from RSV-induced murine fibrosarcoma as the TRA capable of producing protection selectively against RSV-induced tumors but not against RSV-unrelated tumors.[13] Interestingly, analyses have revealed that the above immunochemically defined antigens and TRA in the respective rat[14, 15] and mouse RSV-induced tumor models[13] exhibit an apparent molecular weight of about 60 kD. Since RSV-induced murine tumor cells produce no virus particle, but only the *src* gene product (pp60$^{v\text{-}src}$),[3, 7, 12] the above antigens detected in both systems could represent transformation-associated antigens rather than viral structural antigens such as the *env* gene product. Thus, a critical issue would be to investigate the relation of this TRA to the pp60$^{v\text{-}src}$.

The present study demonstrated that TAA (TRA) expressed on RSV-induced murine fibrosarcoma CSA1M cells represent protein(s) with an apparent molecular weight of about 60 kD but which is distinct from pp60$^{v\text{-}src}$. The distinction between the CSA1M TAA (TRA) and RSV antigens including v-*src* product suggests that the CSA1M TAA (TRA) is a host protein. This finding is also compatible with the observation of Phillips *et al.* which demonstrated the expression of a nonviral, virus strain-specific antigen on ASV-transformed rat cells.[15]

However, it cannot be completely excluded that CSA1M TRA in our system or neoantigens detected in the studies of Phillips *et al.* are the v-*src* product having been so altered as to completely lose the original antigenic determinant and/or tyrosine kinase activity. There is still another possibility that TAA in both models are a composite of viral and host elements as has been reported for the generation of the FOCMA antigen of feline sarcoma virus-transformed cells[9, 26] as an example of virus-host hybrid molecules.

In the above two postulations, the generation of TAA depends on the contribution of the virus-derived gene, especially of v-*src*. This is due to a close relationship between the expression of TAA and acquisition of the transformation phenotype. Alternatively, a more attractive explanation is that the generation of TRA may result from secondary effects, by viral infection or transformation, on a specific host cell molecule. Although the present study has demonstrated the distinction of the CSA1M TRA from functional (kinase activity-positive) and immunologically native pp60$^{v\text{-}src}$, further biochemical characterization will be required to determine the genetic origin of TAA generated during transformation.

Another important aspect of our series of studies is concerned with the properties of TAA as the TRA. Many studies have focused on the detection of TAA as immunochemically defined neoantigens but not as TRA in RSV-induced tumor models. In addition to the present results, our recent studies further revealed that similar TRA molecules exist in various strains of RSV-induced murine fibrosarcoma cells but not in RSV-unrelated tumor cells and function to produce RSV-tumor-specific protection (to be published). Thus, these studies provide the first demonstration of the generation of TRA which is distinct from viral structural gene products or "functional" pp60$^{v\text{-}src}$.

The observation that RSV-tumor-specific TRA is generated in various v-*src*-induced cells again emphasizes the relation of v-*src* to the induction and/or expression of TRA in considering various possibilities, such as that TRA is (a) modified pp60$^{v\text{-}src}$ with altered antigenicity and without the kinase activity, (b) a normally expressed host gene product modified with pp60$^{v\text{-}src}$ or (c) a product of host gene(s) derepressed by v-*src*. Thus, more precise molecular characterization of TRA and its genetic origin could contribute to a better understanding of the role of the viral oncogene in generating TRA, which in turn becomes a target of the host's rejection responses.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Special Project Research-Cancer Bioscience from the Ministry of Education, Science and Culture, Japan. Appreciation is expressed to Dr. H. Hanafusa and Dr. J. Brugge for providing us with the v-*src*-expressing yeast transformant (W303-1A/Yvsrc) and anti-pp60^{src} monoclonal antibody (mAb327), respectively. The authors are also grateful to Miss Kyoko Kajikawa and Miss Yoshiko Aoki for their expert secretarial assistance.

(Received May 10, 1989/Accepted July 25, 1989)

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