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Isolation of Monoclonal Antibodies That Recognize the Transforming Proteins of Avian Sarcoma Viruses

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Thirteen clones of hybrid cells which synthesize antibodies directed against the Rous sarcoma virus (RSV) transforming protein, pp60^{src}, were isolated. Mouse myeloma cells were fused with spleen cells from mice that had been immunized with purified pp60^{src} from bacterial recombinants which direct the synthesis of the RSV *src* gene. The hybridomas which survived the selection medium were screened by immunoprecipitation of pp60^{src} from ^{32}P-labeled lysates of RSV-transformed cells. Monoclonal antibodies produced by subclones derived from 13 hybridomas recognized pp60^{src} encoded by the Schmidt-Ruppin and Prague strains of RSV and the cellular homolog of pp60^{src}. Antibody from clone 261 had a high affinity for the viral *yes* gene product, and antibodies from clones 443 and 463 recognized the transforming proteins encoded by viruses containing the related transforming genes *fps* and *ros*. Several other clones had a low affinity for the viral *yes*, *fps*, and *ros* gene products which could be detected by in vitro phosphorylation of the transforming proteins after immunoprecipitation with the monoclonal antibody. All of the monoclonal antibodies allowed phosphorylation of pp60^{src} and casein in an immune complex-bound reaction.

Rous sarcoma virus (RSV) has proven to be an ideal system to investigate the mechanism of oncogenic transformation by retroviruses. Genetic studies have revealed that a single viral gene product is responsible for the events involved in oncogenic transformation (reviewed in reference 26). The protein product of this gene, designated pp60^{src}, was first identified by immunoprecipitation of radiolabeled lysates of RSV-transformed cells, using antiserum from rabbits bearing tumors induced by RSV (designated TBR serum) (5).

The pp60^{src} protein has been shown to possess a tyrosine-specific kinase activity which has been postulated to be essential for the events involved in oncogenic transformation by RSV (8, 9, 20, 24, 34). Several substrates of pp60^{src}-mediated phosphotransferase have been identified; however, the functional significance of phosphorylation of these substrates remains to be elucidated (4, 6, 10–12, 18, 31, 33).

Uninfected cells contain a gene product which is structurally and functionally analogous to the viral transforming protein, designated pp60$^{c\text{-}src}$ (7, 29, 32). It is believed that the transforming gene of RSV was derived from the cellular gene encoding pp60$^{c\text{-}src}$ (36). The cellular *src* gene product also displays tyrosine-specific phosphotransferase activity (29, 32).

The transforming proteins encoded by several other oncogenic avian and mammalian retroviruses have also been shown to possess tyrosine-specific protein kinase activity (1, 2, 13, 14, 21, 39). Recently, it has been shown that the RSV transforming protein shares considerable amino acid homology with these other tyrosine-kinase transforming proteins (19, 23, 35, 37).

The antiserum from tumor-bearing animals which has been used for the analysis of pp60^{src} is a polyclonal antiserum. Although this antiserum has proven to be invaluable for the identification of this protein and preliminary characterization of its phosphotransferase activity, there are several drawbacks to its use. First, TBR serum contains antibodies to viral structural proteins as well as to pp60^{src}. Second, most of the antisera raised in rabbits infected with the Schmidt-Ruppin (SR) strain of RSV do not recognize pp60^{src} from other strains of RSV or the normal cellular homolog of pp60^{src}, and none of the TBR sera recognize non-*gag*-encoded regions of the transforming proteins from viruses carrying the related transforming genes, *fps*, *yes*, or *ros*. Third, the antigenic determinants recognized by TBR are extremely sensitive to denaturation. Finally, most TBR-derived antibody molecules inactivate the phosphotransferase activity of pp60^{src} on exogenous substrates.

To circumvent many of these problems associated with TBR serum, we have prepared monoclonal antibodies to the pp60^{src} protein. In this study, we report the isolation of 13 hybrid myeloma cell lines producing monoclonal antibodies against pp60^{src}. We have tested these antibodies for precipitation of pp60^{src} derived from other strains of RSV, the transforming proteins encoded by the *fps*, *yes*, and *ros* genes, and the cellular homolog of pp60^{src} in avian and mammalian cells. We have also examined the ability of these antibodies to allow pp60^{src}-mediated phosphorylation of casein.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo fibroblasts were prepared from 11-day-old gs-minus embryos (SPAFAS, Inc., Norwich, Conn.). The Prague (PR) (subgroup A) strain of RSV was obtained from T. Parsons; the SR (subgroup A) strain of RSV and Yamaguchi 73 (Y73) sarcoma virus were obtained from H. Hanafusa; PRCII sarcoma virus was obtained from K. Beemon; and UR-2 sarcoma virus was obtained from P. Balduzzi. SRD-3T3 cells were obtained by infection of BALB-3T3 cells with SRD-RSV, using polyethylene glycol.

Preparation of pp60^{src} for immunization. The *src* gene product was purified from the particulate fraction of *Escherichia coli* cells which carry a plasmid containing the *src* gene fused to a plasmid containing the UV5 *lac* operator-promoter and 24 nucleotides of the β-galactosidase gene. Bacteria from 500 ml of culture medium were lysed, treated with DNase, and solubilized as described previously (15–17). The particulate cellular material was pelleted by centrifugation at 16,000 × *g* for 30 min. (This material was generously provided by Raymond Erikson.) The pellet material was solubilized by boiling for 1 min in electrophoresis sample buffer (16). pp60^{src} was separated from other bacterial proteins by electrophoresis on a 7.5% polyacrylamide gel. pp60^{src} was detected by electrophoresis of ^{32}P-labeled marker pp60^{src} adjacent to the bacterial material. pp60^{src} was eluted from the gel in 0.1% sodium dodecyl sulfate (SDS)–50 mM ammonium carbonate.

Preparation of monoclonal antibodies. A mouse myeloma cell line of BALB/c origin designated X63-Ag8.653 (obtained from the Salk Institute, San Diego, Calif.) was used for the fusions. This line does not express immunoglobin heavy or light chains and therefore permits the generation of hybrids secreting pure monoclonal antibodies. The cells are maintained in culture in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Flow Laboratories, McLean, Va.) and 100 U of penicillin and 100 μg of streptomycin per ml. Frozen stocks were kept in liquid nitrogen in 10% dimethyl sulfoxide–90% fetal calf serum, and cells were thawed approximately 1 week before use for fusion.

Mouse spleen cells. BALB/c mice (3 to 5 weeks old) were injected intraperitoneally three times at weekly intervals with ca. 20 to 50 μg of the purified viral pp60^{src} protein cloned in *E. coli*. The final injection was given intravenously and contained ca. 5 μg of pp60^{src} in phosphate-buffered saline. Four days after the final injection, the mice were bled and sacrificed by cervical dislocation, and their spleens were removed. Spleen cells were then prepared by teasing the spleen apart with needles into RPMI 1640 medium. Erythrocytes were lysed by treatment with 0.83% ammonium chloride, and spleen cells were then counted on a hemacytometer.

Cell fusion was carried out by a modification of the method of Levy and Dilley (25). Briefly, spleen and myeloma cells were pelleted together in a ratio of four spleen cells to one myeloma cell. The pellet was gently suspended in 2 ml of 35% polyethylene glycol (molecular weight; 1,000; Koch-Light, Coinbrook, Buckinghamshire, England) in RPMI 1640 medium, and the cells were immediately centrifuged at 230 × *g* for 6 min. The polyethylene glycol was then aspirated, and the fused cells were suspended in RPMI 1640 medium supplemented with 20% fetal calf serum and 10% NCTC 109 medium (M.A. Bioproducts, Walkersville, Md.). The cells were placed in a T-150 flask and incubated overnight at 37°C under an atmosphere of 5% CO_2–95% air. The following day, hypoxanthine, aminopterin, and thymidine were added to the medium to give a final concentration of 10^{-4} M hypoxanthine, 4 × 10^{-7} M aminopterin, and 1.6 × 10^{-5} M thymidine, and the cells were portioned into 96-well microtiter dishes, allowing ca. 10^5 spleen cells per well. Two weeks after the fusion, medium samples were taken from the wells containing clones and assayed for antibody. Medium from 500 clones was taken and tested for immunoprecipitation of radiolabeled pp60^{src} as described below. Of the 500 clones tested, 16 were found to be positive for precipitation of pp60^{src}. Positive clones were transferred to 24-well microtiter dishes and fed with hypoxanthine-thymidine medium (as above, but omitting the aminopterin). When the cells were sufficiently dense, the media were sampled and again assayed. Clones remaining positive were then grown up, and frozen stocks were made. Cells retained in culture were routinely grown in RPMI 1640 medium with 10% fetal calf serum and antibiotics. To ensure monoclonality and long-term stability of antibody production, strongly positive clones were subcloned by limiting dilution.

Concentration of monoclonal antibodies from medium and preparation of purified IgG. Medium was harvested from hybridoma cells grown at a density of ca. 10^6 cells per ml. This medium was stored under sterile conditions at 4°C until further processing. Concentration of the monoclonal antibodies was performed by precipitation with 60% ultrapure ammonium sulfate (Schwarz/Mann, Orangeburg, N.Y.) and dialysis in one-tenth of the starting volume of phosphate-buffered saline with 0.02% sodium azide. The antibodies were stored at 4°C. Freezing and thawing results in a considerable reduction in the titer of the antibody. Immunoglobulin G (IgG) was prepared from the ammonium sulfate-concentrated medium by the following method. Concentrated medium (10 ml) was incubated for 2 h at 4°C with 200 μl of swollen protein A-Sepharose beads (Sigma Chemical Co., St. Louis, Mo.). The beads were then washed three times with 100 mM Tris-hydrochloride (pH 8). IgG was eluted by incubation with 0.5 ml of 100 mM glycine (pH 3). After sedimentation of the beads, the supernatant fraction was neutralized immediately with 1 M Tris-hydrochloride (pH 8.0). IgG was stored at 4°C and was never subjected to freezing and thawing. The final concentration of IgG was less than 0.1 μg/μl.

Sera. Monoclonal antibody to p19 was obtained from David Boettiger. TBR serum was prepared as described previously (5) from a rabbit bearing a tumor induced by RSV. This antiserum contains antibody to pp60^{src} as well as to the *gag* gene products. Rabbit antiserum directed against the *E. coli*-produced pp60^{src} protein was a generous gift from R. L. Erikson (16).

Immunoprecipitation. For the preparation of animal cell extracts, cultures were labeled for 4 h with $^{32}P_i$ (0.5 mCi/ml, carrier-free; ICN Pharmaceuticals Inc., Irvine, Calif.) in phosphate-free medium. Cells were lysed and clarified as described previously (5). Either ammonium sulfate-concentrated media or purified IgG from the hybridoma cell lines were utilized for immunoprecipitation of radiolabeled lysates. Lysates were incubated 45 min with each monoclonal antibody, 20 min with goat anti-mouse IgG (Meloy Laboratories, Springfield, Va.), and precipitated with the protein A-containing bacteria, *Staphylococcus aureus* (22). The bacterial-bound immune complex was washed three times with radioimmunoprecipitation (RIPA) buffer (5), and the immunoprecipitated proteins were eluted and analyzed on 7.5% SDS-polyacrylamide gels.

Detection of phosphotransferase activity. i. Phosphorylation of casein. Protein kinase activity was assayed by phosphorylation of casein. pp60^{src} bound to *S. aureus* which had been immunoprecipitated with monoclonal antibodies was suspended in 5 mM $MgCl_2$–20 mM Tris-chloride (pH 7.2) and incubated with 5 μg of casein (Sigma). After the addition of 10 μCi of [γ-^{32}P]ATP (ICN), the reaction was incubated at 4°C for 20 min. The reaction was terminated by the addition of SDS-sample buffer and subjected to electrophoresis on 10% SDS-polyacrylamide gels followed by autoradiography to determine the phosphorylation of casein.

ii. In vitro phosphorylation of pp60^{src}, pp90^{yes}, pp110^{fps}, and pp68^{ros}. In vitro phosphorylation of pp60$^{c\text{-}src}$ was assayed by utilizing pp60$^{c\text{-}src}$ bound to *S. aureus* which had been precipitated from a lysate prepared from a 12-day embryonic chicken brain as described above. The immune complex was suspended in 5 mM $MgCl_2$–20 mM Tris-chloride (pH 7.2)–10 μCi of [γ-^{32}P]ATP and allowed to incubate at 4°C for 20 min. The precipitates were washed once with RIPA medium and then suspended in SDS-sample buffer and subjected to electrophoresis on 7.5% SDS-polyacrylamide gels followed by autoradiography to determine phosphorylation of pp60^{src}. A similar procedure was utilized for detection of phosphorylation of the transforming proteins from Y73-, PRCII-, or UR-2-transformed chicken cells, except that 5 mM $MnCl_2$ replaced $MgCl_2$.

RESULTS

We isolated 13 hybridoma cell lines producing antibodies which recognize pp60^{src}. These cell lines were prepared by fusion of myeloma cells with spleen cells of BALB/c mice immunized with the *src* protein extracted from *E. coli* cells carrying a cloned *src* gene. Five hundred clones survived the selection procedure, and medium from each clone was screened for antibody directed against pp60^{src} by immunoprecipitation of lysates from ^{32}P-labeled PR-RSV-transformed chicken cells. Sixteen wells contained clones

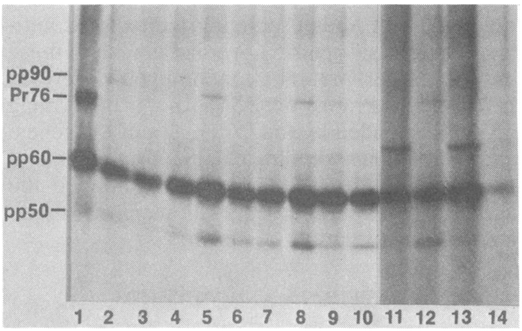

FIG. 1. Proteins immunoprecipitated from $^{32}P_i$-labeled Prague RSV-transformed chicken cells. A lysate from a 100-mm culture of ^{32}P-labeled Prague RSV-transformed chicken cells was immunoprecipitated with 5 μl of TBR serum (lane 1) or 100 μl of concentrated medium from hybridoma cells as described in the text. Lane 2, monoclonal antibody 69; lane 3, 78; lane 4, 127; lane 5, 191; lane 6, 199; lane 7, 200; lane 8, 261; lane 9, 273; lane 10, 327; lane 11, 443; lane 12, 450; lane 13; 463; lane 14, 492.

positive for pp60^{src} precipitation. All 16 clones were subcloned by limiting dilution to assure the monoclonality of each hybridoma. Of the original 16 clones, only 3 clones have ceased producing antibody molecules which recognize pp60^{src}.

Figure 1 displays an autoradiogram of a gel containing the $^{32}P_i$-labeled proteins immunoprecipitated from Prague RSV-transformed chicken cells, using TBR serum (lane 1) or concentrated medium from the antibody-producing hybridoma cells (lanes 2 through 14). TBR serum immunoprecipitated pp60^{src} as well as Pr76, the initial translation product of the *gag* gene, and two cellular proteins of M_r 90,000 (90K) and 50K which are associated in a complex with a small percentage of pp60^{src} molecules (3, 28). The monoclonal antibodies immunoprecipitated pp60^{src} as well as the two cellular proteins pp90 and pp50. Antibodies 443 and 463 (lanes 11 and 13) consistently precipitated less pp90 and pp50 relative to pp60^{src}, suggesting that pp90 and pp50 may interfere with recognition of the determinants recognized by these antibodies. Antibodies 443 and 463 also precipitated another ^{32}P-labeled protein of M_r 68K which was found to contain phosphoserine and phosphotyrosine (data not shown). The identity of this protein is under investigation. All of the monoclonal antibodies which recognized pp60^{src} from cells infected with PR-RSV also recognized SR virus-encoded pp60^{src} (including mutant viruses derived from the PR and SR strains of RSV which carry temperature-sensitive defects in the *src* gene; data not shown). The identity of pp60$^{v\text{-}src}$ was confirmed by partial proteolytic cleavage with V8 protease (data not shown).

Cross-reaction with cellular pp60^{src}. Figure 2A displays the immunoprecipitation of ^{32}P-labeled proteins from uninfected chicken cells. IgG from all of the hybridomas precipitated a protein which comigrated with the 60K protein precipitated with serum directed against the pp60^{src} protein expressed in *E. coli* (16). The identity of these proteins as pp60^{src} was confirmed by partial proteolytic peptide analysis with V8 protease (data not shown). In addition, monoclonal antibodies 273 and 327 (lanes 7 and 8) were found to specifically immunoprecipitate the human and mouse cellular *src* proteins (data not shown; other antibodies not tested).

Figure 2B displays the phosphorylation of pp60$^{c\text{-}src}$ after immunoprecipitation with IgG purified from the hybridoma medium. This assay was performed with a highly concentrated lysate from fresh chicken brain tissue and appears to be a more sensitive assay of pp60$^{c\text{-}src}$ than immunoprecipitation of ^{32}P-labeled chicken cell lysates. All of the monoclonal antibodies precipitated 60K protein which was phosphorylated after the addition of [γ-^{32}P]ATP to the immunoprecipitated proteins (antibody 199 not shown). The identity of this 60K protein which was phosphorylated in this assay as pp60$^{c\text{-}src}$ was confirmed by partial proteolytic peptide analysis with V8 protease. These results suggest that all of the monoclonal antibodies recognize determinants which are shared with pp60$^{c\text{-}src}$. In both assays shown in Fig. 2, antibodies 273 and 327 precipitated higher levels of pp60^{src} than did the other monoclonal antibodies.

Cross-reaction with other viral transforming proteins. Recent investigations have found that the protein products of the transforming genes of other avian sarcoma viruses bear considerable amino acid sequence homology with pp60^{src} (19, 23, 35, 37). To determine whether any of the monoclonal antibodies to pp60^{src} recognize these related proteins, we examined lysates from chicken cells infected with avian sarcoma viruses containing the *yes*, *fps*, and *ros* genes. These viruses included Yamaguchi 73 (Y73) (21), UR-2 (14), and PRCII (27), which encode transforming proteins of 90K (pp90^{yes}), 68K (pp68^{ros}) and 110K (pp110^{fps}) molecular weight, respectively. These transforming proteins are *gag* gene fusion products, and antibody which recognizes the *gag* portion was used as a positive control in these experiments.

Figure 3 shows the immunoprecipitation of ^{32}P-labeled proteins from Y73-transformed chicken cells. It can be seen that antibody 261 (lane 8) precipitated greater levels of pp90^{yes} than did monoclonal antibody to p19 (lane 1), the *gag*-encoded protein. Three other monoclonal antibodies (443, 450, and 492; lanes 11, 12, and 13, respectively) precipitated lesser amounts of pp90^{yes}.

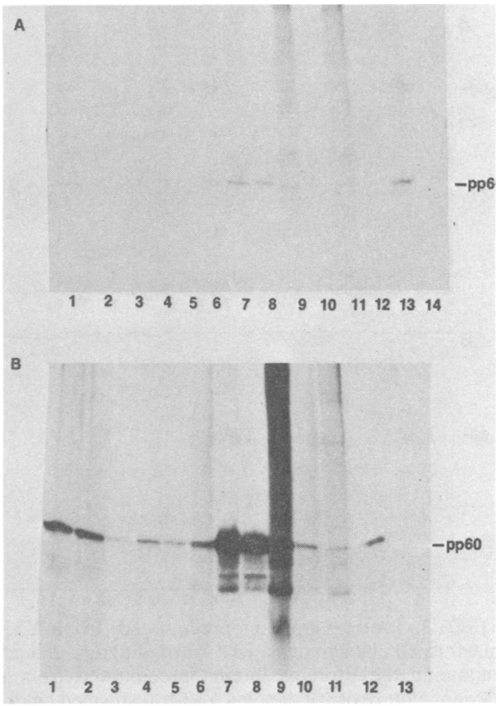

FIG. 2. Proteins immunoprecipitated from $^{32}P_i$-labeled normal chicken cells. (A) Autoradiogram of a gel containing the proteins immunoprecipitated from a lysate prepared from three 100-mm cultures of $^{32}P_i$-labeled normal chicken cells with 20 μl of IgG from hybridoma cell line 69 (lane 1), 78 (lane 2), 127 (lane 3), 191 (lane 4), 200 (lane 5), 261 (lane 6), 273 (lane 7), 327 (lane 8), 443 (lane 9), 450 (lane 10), 463 (lane 11), 492 (lane 12), anti-bacterial pp60^{src} (lane 13), or antimouse IgG alone (lane 14). (B) Proteins phosphorylated in vitro after immunoprecipitation of a lysate of chicken brain with purified IgG as described in the text. Lane 1, monoclonal antibody 69; lane 2, 78; lane 3, 127; lane 4, 191; lane 5, 200; lane 6, 261; lane 7, 273; lane 8, 327; lane 9, 443; lane 10, 450; lane 11, 463; lane 12, 492; lane 13, antimouse IgG alone.

To determine whether the monoclonal antibodies which were negative for pp90^{yes} precipitation in the above experiment could indeed recognize pp90^{yes} in a more sensitive assay, we performed an in vitro phosphorylation reaction. Figure 3B demonstrates the autophosphorylation of pp90^{yes} bound to the monoclonal antibodies. Unlabeled lysates of Y73-transformed chicken cells were immunoprecipitated with the monoclonal antibodies followed by incubation with [γ-^{32}P]ATP and $MnCl_2$. In this in vitro reaction, pp90^{yes} was phosphorylated. Precipitation with monoclonal antibody to p19 and 261 (lanes 1 and 8) allowed the greatest level of pp90^{yes} phosphorylation; however, lesser amounts of phosphorylation were detected after precipitation of monoclonal antibodies 443, 450,

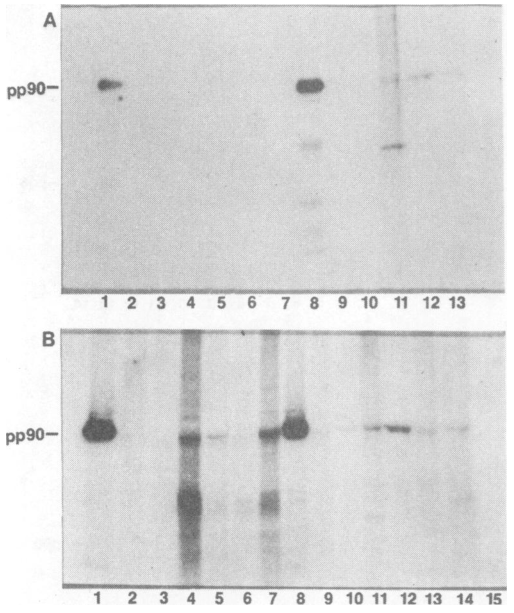

FIG. 3. Proteins immunoprecipitated from Y73-transformed chicken cells. (A) Autoradiogram of a gel containing the proteins immunoprecipitated from a 100-mm culture of $^{32}P_i$-labeled Y73-transformed chicken cells as described in the text. Lane 1, anti-p19 antibody; lane 2, concentrated medium from hybridoma 69; lane 3, 78; lane 4, 111; lane 5, 127; lane 6, 191; lane 7, 200; lane 8, 261; lane 9, 273; lane 10, 327; lane 11, 443; lane 12, 450; lane 13, 492. (B) Autoradiogram of a gel containing the proteins phosphorylated after immunoprecipitation from a lysate of Y73-transformed chicken cells as described in the text. Lane 1, anti-p19 antibody; lane 2, concentrated medium from monoclonal antibody 69; lane 3, 78; lane 4, 127; lane 5, 191; lane 6, 199; lane 7, 200; lane 8, 261; lane 9, 273; lane 10, 327; lane 11, 443; lane 12, 450; lane 13, 463; lane 14, 492; lane 15, antimouse antibody alone.

and 492 (lanes 11, 12, and 14, respectively). The identity of these proteins as pp90^{yes} was confirmed by peptide analysis with V8 protease (data not shown). The protein migrating slightly faster than pp90^{yes} in lanes 4, 5, and 7 did not share any peptides with pp90^{yes}, whereas proteins precipitated by antibodies 261, 443, 450, and 492 were identical to pp90^{yes}.

Since the in vitro reaction described above for Y73-transformed chicken cells proved to be more sensitive than the direct immunoprecipitation of ^{32}P-labeled lysates, the autophosphorylation reaction was utilized for determining cross-reactivity with other viral transforming proteins. Figure 4A shows the autophosphorylation of the PRCII transforming protein, pp110^{fps}, bound to the monoclonal antibodies. Antibodies 443 and 463 (lanes 10 and 12) precipitated a 110K protein which comigrated with the pp110^{fps} protein precipitated by monoclonal antibody to p19 (lane 13) and which was identical to pp110^{fps} by partial proteolytic peptide analysis with V8 protease (data not shown). The phosphorylated protein of M_r 60K did not show any partial peptides identical to those of pp60$^{c\text{-}src}$. Longer exposure of this gel revealed faint protein bands in other lanes which comigrated with pp110^{fps}. This experiment was repeated with more monoclonal antibody and a more highly concentrated lysate of PRCII-infected chicken cells to increase the sensitivity of this assay. Under these conditions, several monoclonal antibodies precipitated a 110K protein which was identical to pp110^{fps} by peptide analysis. The level of phosphorylation of pp110^{fps} was consistently at least

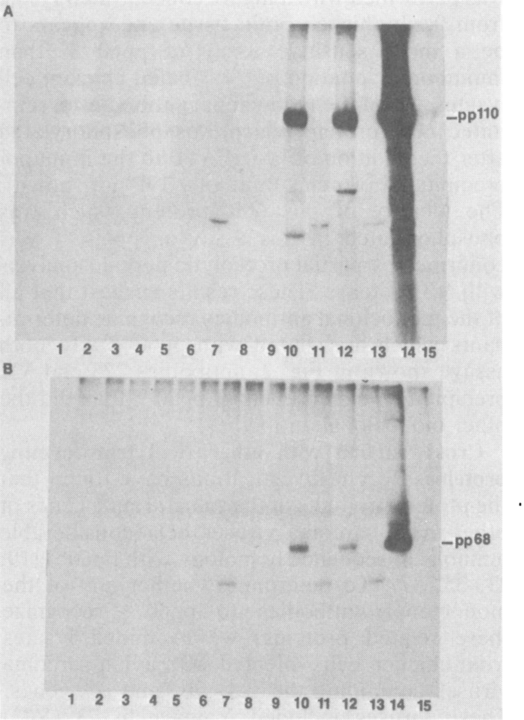

FIG. 4. Proteins immunoprecipitated from PRCII- or UR-2-transformed chicken cells. (A) Autoradiogram of a gel containing the proteins phosphorylated in vitro after immunoprecipitation from a lysate of PRCII-transformed chicken cells as described in the text. Lane 1, purified IgG from hybridoma 69; lane 2, 78; lane 3, 127; lane 4, 191; lane 5, 199; lane 6, 200; lane 7, 261; lane 8, 273; lane 9, 327; lane 10, 443; lane 11, 450; lane 12, 463; lane 13, 492; lane 14, anti-p19 antisera; lane 15, antimouse IgG alone. (B) Autoradiogram of a gel containing the proteins phosphorylated after immunoprecipitation from a lysate of UR-2-transformed chicken cells as described in the text. Lane 1, purified IgG from hybridoma 69; lane 2, 78; lane 3, 127; lane 4, 191; lane 5, 199; lane 6, 200; lane 7, 261; lane 8, 273; lane 9, 327; lane 10, 443; lane 11, 450; lane 12, 463; lane 13, 492; lane 14, TBR antiserum; lane 15, antimouse IgG alone.

10-fold less than that found with antibodies 443 and 463, and the relative levels of phosphorylation varied for each monoclonal antibody in multiple experiments. The 110K protein band detected in the control reaction of Fig. 4A was not related to pp110^{fps} and was not reproducibly detected in other experiments. These results suggest that some of the monoclonal antibodies have a low affinity for pp110^{fps} and that these reactions are carried out at the lower limit of detection. When PRCII cells are labeled with $^{32}P_i$, pp110^{fps} can only be detected with antibodies 443 and 463.

Figure 4B shows a similar in vitro phosphorylation of the UR-2 transforming protein, pp68^{ros}, bound to the monoclonal antibodies. Antibodies 443, 463, and 492 (lanes 10, 12, and 13) precipitated a 68K protein which comigrated with pp68^{ros} precipitated with TBR serum. TBR serum contains antibodies to *gag*-specific determinants of pp68^{ros} and was used to precipitate pp68^{ros} because the monoclonal antibody to p19 did not precipitate pp68^{ros} efficiently. The identity of pp68^{ros} was corroborated by peptide analysis with V8 protease. We also found low levels of precipitation of pp68^{ros} with monoclonal antibodies other than 443 and 463 when this assay was performed under the more sensitive conditions described above for pp110^{fps}. pp68^{ros} could also be detected by immunoprecipitation of [^{35}S]methionine-labeled lysates of UR-2-infected chicken cells (data not shown).

Protein kinase activity. To determine whether any of the monoclonal antibodies interfere with the phosphotransferase activity of pp60^{src}, protein kinase activity was assayed by phosphorylation of exogenous casein in an immune complex-bound reaction (Fig. 5A). An identical plate of ^{32}P-labeled cells was immunoprecipitated with the same amount of IgG to demonstrate the relative amount of pp60^{src} precipitated with each monoclonal antibody (Fig. 5B). Figure 5A shows phosphorylation of casein after immunoprecipitation of unlabeled lysates of SRD-RSV-transformed 3T3 cells. It can be seen that pp60^{src} bound to all of the monoclonal antibodies was able to phosphorylate casein. The relative levels of casein phosphorylation generally reflect the level of ^{32}P-labeled pp60^{src} shown in Fig. 5B. The protein of M_r 60K which was phosphorylated in the in vitro reaction (Fig. 5A) is pp60^{src}. Differences in the level of phosphorylation of pp60^{src} also appear to reflect the ability of the monoclonal antibody to precipitate ^{32}P-labeled pp60^{src}. Although antibodies 273 and 327 (lanes 7 and 8) allowed phosphorylation of casein to a lesser extent than the other monoclonal antibodies, this was not reproducible in other experiments. Monoclonal antibodies 127, 199, and 200 showed a similar phosphorylation of pp60^{src} and casein (data not shown). The TBR serum used in this assay to precipitate pp60^{src} also allowed casein phosphorylation. IgG was not phosphorylated after immunoprecipitation of pp60^{src} by any of the monoclonal antibodies. In a similar experiment with lysates from Y73-transformed chicken cells, it was found that pp90^{yes} which was immunoprecipitated with antibody 261 also phosphorylated casein.

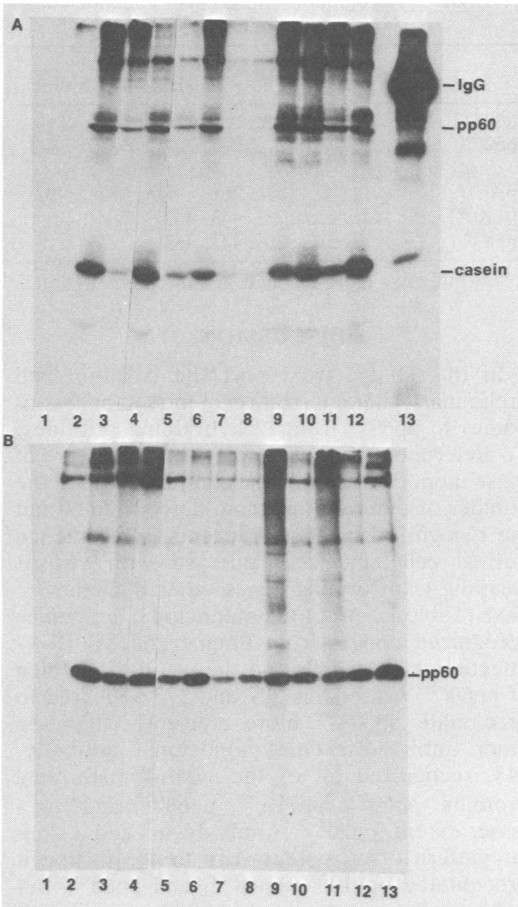

FIG. 5. Phosphorylation of casein by pp60^{src} bound to monoclonal antibodies. (A) Autoradiogram of a gel representing the phosphorylation of casein after immunoprecipitation of pp60^{src} from SR-D-transformed 3T3 cells as described in the text. Lane 1, antimouse IgG alone; lane 2, purified IgG from hybridoma 69; lane 3, 78; lane 4, 191; lane 5, 200; lane 6, 261; lane 7, 273; lane 8, 327; lane 9, 443; lane 10, 450; lane 11, 463; lane 12, 492; lane 13, TBR-B antiserum. (B) Autoradiogram of a gel containing the proteins immunoprecipitated from an equivalent amount of a $^{32}P_i$-labeled SRD-3T3 cell lysate as that used in (A) Lane 1, antimouse IgG alone; lane 2, purified IgG from hybridoma 69; lane 3, 78; lane 4, 191; lane 5, 200; lane 6, 261; lane 7, 273; lane 8, 327; lane 9, 443; lane 10, 450; lane 11, 463; lane 12, 492; lane 13, TBR-B antiserum.

TABLE 1. Recognition of proteins by monoclonal antibodies

Protein	Recognized by monoclonal antibodies[a]
pp60$^{v\text{-}src}$	All
pp60$^{c\text{-}src}$	273, 327 > 69, 78, 443, 463 > other
pp90^{yes}	261 > 443, 450 > 492
pp110^{fps}	443, 463
pp68^{ros}	443, 463

[a] > Indicates more efficient immunoprecipitation.

DISCUSSION

In this study, we report the isolation and preliminary characterization of monoclonal antibodies to pp60^{src} from 13 hybridoma cell lines. To determine the extent of cross-reactivity of these monoclonal antibodies and to estimate the number of different antigenic determinants that are recognized by these reagents, we screened normal cells and cells infected with viruses carrying transforming genes other than the *src* gene (Table 1). All of the monoclonal antibodies recognized pp60^{src} from Prague and SR RSV-infected chicken cells and the cellular homolog of pp60^{src}. Antibodies 327 and 273 appeared to precipitate pp60$^{c\text{-}src}$ more efficiently than the other antibodies. One monoclonal antibody, 443, recognized all of the viral transforming proteins: pp60^{src}, pp110^{fps}, pp68^{ros}, and, to a lesser extent, pp90^{yes}. Antibody 463 had a similar pattern of cross-reactivity to 443 in that it precipitated pp110^{fps}, pp68^{ros}, and pp60^{src}, but we have not yet detected pp90^{yes} precipitation by 463. This suggests that these antibody molecules do not recognize the same epitope on pp60^{src}. It is noteworthy that both antibodies precipitated the same ^{32}P-labeled 68K protein from RSV-transformed cell lysates (Fig. 1, lanes 11 and 13).

Several of the monoclonal antibodies appeared to have weak cross-reactivity with pp90^{yes}, pp110^{fps}, and pp68^{ros} that was detectable in an in vitro phosphorylation assay but was not detectable by immunoprecipitation of proteins radiolabeled in vivo. It is not clear whether this weak recognition is due to amino acid sequence differences or to different conformational arrangements of each of the epitopes in the various transforming proteins. We are presently attempting to vary the conditions of immunoprecipitation to optimize for recognition of these proteins by the different monoclonal antibodies. Preliminary experiments comparing the efficiency of precipitation of the pp60^{src} protein expressed in *E. coli* with that of pp60^{src} from RSV-transformed chicken cells indicates that several of the monoclonal antibodies recognize the *E. coli*-produced protein much more efficiently than the protein from transformed chicken cells. That is not an unexpected result since the antigen used for immunization was a denatured form of the bacterial protein. This protein is distinct from the RSV *src* protein in that it contains eight amino acids from β-galactosidase at its amino end. Since all of the monoclonal antibodies recognized a 52K cleavage product of pp60^{src} which has lost the amino terminal sequences of pp60^{src} (data not shown), the monoclonal antibodies would not appear to recognize the β-galactosidase-derived sequences. Therefore, differences in the precipitation of the two forms of pp60^{src} could reflect differences in the conformation of pp60^{src} molecules derived from either the bacterial or the animal cell environment.

This battery of monoclonal antibodies should be valuable for investigations of pp60^{src}, its normal cellular homolog, and the related transforming proteins from other avian sarcoma viruses. Monoclonal antibodies provide the technology to perform immunodetection assays without contaminating reactivities which are present in polyclonal serum. Parsons and coworkers have previously reported the isolation of a monoclonal antibody which recognizes pp60$^{v\text{-}src}$ (30). Monoclonal antibodies 273 and 327 will considerably improve the means for analysis of the normal cell *src* protein since most sera from animals bearing RSV-induced tumors either do not recognize pp60$^{c\text{-}src}$ or have a low titer of antibodies to this protein. None of the RSV-specific TBR sera which have been described previously recognize transformation-specific regions of the viral *fps*, *ros*, or *yes* gene products. With the exception of pp130^{fps} (13), these proteins have been characterized by using antibodies directed against the *gag*-specific region of these proteins. Monospecific antibody directed against the transformation-specific regions of these proteins will make it possible to focus on the transforming protein without the additional recognition of the *gag* gene products. Large-scale purification of these antigens is possible by using the monoclonal antibodies for immunoaffinity chromatography.

All of the monoclonal antibodies described in this report allowed phosphorylation of casein in an immune complex-bound assay. This provides a rapid and simple means of assaying phosphorylation of exogenous substrates. This also provides an assay which can be used to investigate the normal cellular *src* protein from unlabeled material. We have not yet determined whether the *src* protein from lower eucaryotes can be recognized by any of the monoclonal antibodies.

From the preliminary analysis of the cross-reactivities of these monoclonal antibodies, we can predict that this battery of antibodies recognizes at least six different antigenic determinants. None of these determinants appears to be within the amino-terminal 8,000 daltons of $pp60^{src}$ since all of the antibodies recognized the 52K cleavage product of $pp60^{src}$ generated during lysis of RSV-transformed cells (data not shown). Since all of the monoclonal antibodies recognized the cellular homolog of $pp60^{src}$, they apparently do not recognize the carboxyl-terminal 12 amino acids which are unique to $pp60^{v\text{-}src}$ (38). Finer-detailed mapping and competition assays are required to distinguish other specificities.

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