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Formation of an Infectious Virus-Antibody Complex with Rous Sarcoma Virus and Antibodies Directed Against the Major Virus Glycoprotein

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Preparations of Rous sarcoma virus (RSV) can form an infectious viral-antibody complex with antibodies raised against the major glycoprotein, gp85, isolated from avian myeloblastosis virus and Prague-RSV subgroup C. Binding of anti-gp85 antibodies to RSV can be demonstrated by the inhibition of focus-forming activity after addition of goat anti-rabbit immunoglobulin and by a shift in density of virions treated with anti-gp85 serum. Group- rather than subgroup-specific regions of viral gp85 appear to be the site of binding for infectious complex.

The major glycoprotein of the avian tumor viruses has been identified as a polypeptide of 85,000 mol wt (1, 4, 5, 10). This protein, called gp85, appears to be essential for virus to infect host cells, and regions of this protein contain the type- or subgroup-specific determinants of the avian oncornaviruses (4, 11, 15, 17, 20). Antibodies have been prepared against a purified form of gp85 isolated from avian myeloblastosis virus and Prague-Rous sarcoma virus subgroup C (Pr-RSV-C) (6, 16). The preparation of rabbit anti-gp85 antibodies used in this work was carried out in the laboratory of D. P. Bolognesi, Duke University.

The rabbit antibodies prepared against purified gp85 do not show a strong neutralizing effect on the biological activity of RSV (16) (Table 1). I have found, however, that these anti-gp85 antibodies can bind effectively to intact RSV and that this virus-antibody complex is infectious. The infectivity of the virus-antibody complex can be demonstrated by comparing the titer of RSV in a focus assay after the virus has been treated first with anti-gp85 serum and then with goat anti-rabbit immunoglobulin (IgG) serum. The addition of goat anti-rabbit IgG to RSV pretreated with anti-gp85 serum consistently decreased RSV titers from five- to tenfold (Table 1). In the absence of the goat anti-rabbit IgG there was very little neutralization of Pr-RSV by anti-gp85 serum, and the presence of a precipitate formed between a nonimmunized (control) rabbit serum and goat anti-rabbit IgG had a negligible effect on virus titer (Table 1). Table 1 also shows the effect of increasing the concentration of anti-gp85 antiserum on Pr-RSV-B titers. At the highest concentration, 96% of infectious virus appears to be complexed with antibody.

The antigenic determinants of gp85 that produce these "complexing" antibodies are not in those regions of the protein involved in subgroup or type specificity. Antibodies raised against gp85 from Pr-RSV-C or avian myeloblastosis virus (subgroup B) react equally well in forming a complex with Pr-RSV-C (Table 1). Anti-gp85 raised against Pr-RSV-C was also equally effective in complexing samples of Prague A, B, and C viruses (data not shown). I also compared the extent of infectious complex formation between anti-gp85 (Prague C) and Prague C virus grown in both duck and chicken embryo fibroblasts. Both virus samples showed a similar fivefold drop in titer after treatment with anti-gp85 serum and goat anti-rabbit IgG.

Two recent reports provide additional evidence that these rabbit anti-gp85 antibodies recognize group-specific regions of RSV (7, 16), and earlier experiments using disrupted preparations of RSV also suggested a group specificity for anti-gp85 antiserum (1). Several other antibodies have been prepared against isolated proteins of avian tumor virus (2), and I tested these for infectious complex formation. Antibodies against gp37, p27, p19, p15, p12, and p10 failed to form infectious complexes with Prague A RSV: no decrease in titer was observed after incubation with any of these antibodies. Anti-gp37 serum was tested at concentrations five

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TABLE 1. *Effect of rabbit anti-gp85 serum and goat anti-rabbit IgG on focus-forming ability of RSV*[a]

Virus strain	Serum	FFU/ml × 10^{6}
Prague A	Control serum + saline	1.5
	Control serum + goat anti-rabbit	1.4
	Anti-$gp85_{AMV}$ + saline	1.0
	Anti-$gp85_{AMV}$ + goat anti-rabbit	0.2
Prague B	Anti-$gp85_{AMV}$ (1:6) + saline	1.0
	Anti-$gp85_{AMV}$ (1:120) + goat anti-rabbit	0.5
	Anti-$gp85_{AMV}$ (1:60) + goat anti-rabbit	0.15
	Anti-$gp85_{AMV}$ (1:20) + goat anti-rabbit	0.1
	Anti-$gp85_{AMV}$ (1:6) + goat anti-rabbit	0.04
Prague C	Control serum + goat anti-rabbit	1.5
	Anti-$gp85_{PRC}$ + saline	1.5
	Anti-$gp85_{PRC}$ + goat anti-rabbit	0.10
	Anti-$gp85_{AMV}$ + goat anti-rabbit	0.10

[a] Virus (~10^{6} focus-forming units [FFU]/ml) was incubated with rabbit antiserum in a total volume of 0.1 ml at 37 C for 60 min; then either 0.1 ml of goat anti-rabbit IgG (Gateway Immunosera Co., Cahokia, Ill.) or 0.1 ml of phosphate-buffered saline was added, and the tubes were incubated an additional 60 min at 37 C. Eagle medium containing 1% fetal calf serum and 1% heated chicken serum was added to bring the volume to 1.0 ml. Dilutions were made in this medium, and focus assays were determined according to the procedure of Weiss (20). For assay of Pr-RSV-B and -C, 4 μg of polybrene per ml was included in the dilutions to ensure better adsorption of virus. In experiments with Pr-RSV-A and -C viruses, the anti-gp85 serum was used at a final dilution of 1:60; in Pr-RSV-B the numbers in parentheses refer to the dilution of the anti-gp85 serum used in the initial 60-min incubation. A fivefold excess of goat anti-rabbit IgG was used in all experiments. AMV, Avian myeloblastosis virus; PRC, Pr-RSV-C.

times that of the anti-gp85 serum. Anti-p19, anti-p12, and anti-p19 sera were tested at concentrations twice that of anti-gp85 serum, and anti-p19 serum was also tested against Pr-RSV-B.

A direct test for the binding of anti-gp85 antibodies to intact virions was carried out by adding antiserum to a preparation of virus that had been harvested from Pr-RSV-B-transformed cells labeled with [^{35}S]methionine and [^{3}H]glucosamine. After addition of antiserum, the preparation was centrifuged to equilibrium in a 20 to 50% sucrose density gradient, and the peak of radioactive virus was tested for radioimmune precipitation with goat anti-rabbit IgG. The results of this experiment showed that the preparation of virus treated with anti-gp85 antiserum banded at a density of 1.177, and samples from fractions across the peak of radioactive virus were precipitated with goat anti-rabbit IgG (Fig. 1A). Virus that had been mixed with a nonimmunized control rabbit serum banded at a density of 1.169, and no radioactivity was recovered in immunoprecipitates formed by adding goat anti-rabbit IgG to fractions from the peak of the virus from this gradient (Fig. 1B). Thus, binding of anti-gp85 antibodies to intact virus is detectable by both the biological assay noted in Table 1 and by a shift in density of viral particles that have been mixed with antiserum.

Further analyses were carried out with the radioimmune precipitates obtained from the virus that had been mixed with anti-gp85 serum prior to centrifugation (Fig. 1A, virus peak). The resuspended precipitate was heated in 2% sodium dodecyl sulfate containing 0.5 M mercaptoethanol and electrophoresed in 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (18). Slices of 1-mm thickness were made and incubated at 37 C for 16 h with 0.5 ml of water. The pattern of labeled proteins from this precipitate was compared with a sample of the virus taken directly from the sucrose gradient peak (Fig. 1A) but not precipitated with goat anti-rabbit IgG. Two major peaks of ^{3}H were found in both the immunoprecipitated and nonprecipitated samples (hatched areas of Fig. 2A and 2B, respectively). The larger is gp85, and the smaller is most likely the gp37 of RSV. The ratio of gp85-gp37 is the same for both samples. Three major peaks of [^{35}S]protein were present in the gel pattern of the nonprecipitated sample (Fig. 2B). I assign the peak near the bottom of the gel to RSV p15, p12, and p10 (6) and the other peaks to p27 and p19 (arrow, Fig. 2). Only one of the major peaks, that assigned to p19, is found in the immunoprecipitated sample (Fig. 2A), and most of the p27, p15, p12, and p10 have been removed. Prior

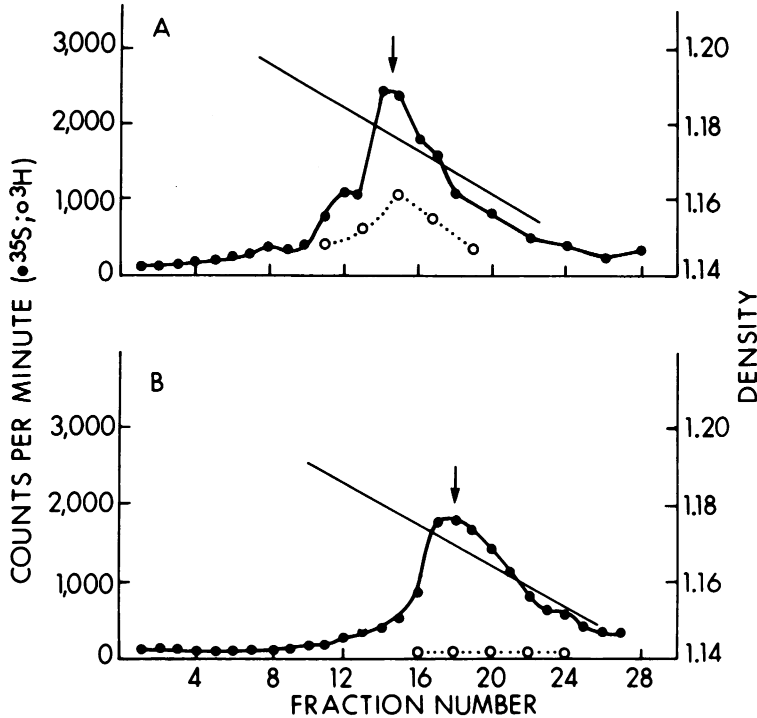

FIG. 1. *Sucrose density gradient centrifugation of Pr-RSV-B after mixing with rabbit sera. Virus was harvested from media of RSV-transformed chicken embryo fibroblasts by centrifugation in an SW40 MSE rotor for 2 h at 35,000 rpm. Pellets of virus were resuspended in 0.4 ml of Eagle minimal medium and 0.2-ml samples mixed with either 0.05 ml of anti-gp85 or normal rabbit serum. After a 60-min incubation at 37 C, the samples were diluted to 1.0 ml with a buffer consisting of 0.01 M Tris-hydrochloride (pH 7.4), 0.1 M sodium chloride, 10^{-3} M EDTA, 0.1% bovine serum albumin, and 1% dimethyl sulfoxide and layered onto a linear 20 to 50% sucrose gradient prepared with the same buffer. Centrifugation was in an SW40 MSE rotor at 35,000 rpm for 2 h. Fractions of 0.2 ml were collected, and 0.02 ml was analyzed for ^{35}S-radioactivity. For radioimmune precipitation, 0.1-ml samples from the peak fractions of the gradient were mixed with 0.05 ml of goat anti-rabbit IgG serum and 0.01 ml of normal rabbit serum. Precipitates formed after incubation at 4 C for 16 h and were washed three times with buffer containing 2% Nonidet P-40 and 1 mg of bovine serum albumin per ml in phosphate-buffered saline. Washed precipitates were solubilized in buffer containing 2% sodium dodecyl sulfate and analyzed for ^{3}H after precipitation with trichloroacetic acid. For labeling of virus, 0.5 mCi of [6-^{3}H]glucosamine (Amersham Radiochemical, 19.6 Ci/mM) was added to a 90-mm dish of cells in 5 ml of Eagle minimal medium containing one-fourth the normal amount of glucose and supplemented with 1% fetal calf serum and 1% heated chicken serum. Six hours after addition of the label, the medium was removed and replaced with nonradioactive medium. After an additional 3 h, media were collected. A third harvest was obtained after another 3-h incubation. Only 3-h harvests were analyzed, and these were combined with several samples of 3-h virus harvested from unlabeled cells. [^{35}S]methionine (0.2 mCi, 300 Ci/mM) was added to a 90-mm dish containing Eagle minimal media lacking methionine. Unlabeled methionine was added to 10^{-6} M (final concentration), and two 3-h virus harvests were obtained. The ^{3}H- and ^{35}S-labeled viruses were mixed prior to initial centrifugation. (A) Virus mixed with anti-gp85 antibodies; (B) virus mixed with normal serum.*

to resuspending the precipitate for gel analysis, it had been washed three times with 2% Nonidet P-40, a nonionic detergent that disrupts RSV cores from the virus envelope (3). The results shown in Fig. 2A indicate that the core proteins (p10, p12, p15, and p27) were washed free from the immunoprecipitate by Nonidet P-40, and radioactivity measurements of the first Nonidet P-40 wash have confirmed this. This loss of ^{35}S label can explain an observed decrease in the ^{35}S-^{3}H ratio from a value of 2.2 in the protein of the original sample of the gradient peak to 0.5 in the resuspended precipitated proteins. The amount of [^{3}H]protein recovered in the precipitate after washing was 30% of the material added to the radioimmune precipitation reaction.

The presence of both RSV glycoproteins in association with an anti-gp85 anti-rabbit IgG immunoprecipitate is not unexpected, because gp85 and gp37 have been observed to co-precipitate with anti-gp85 serum (16). Somewhat sur-

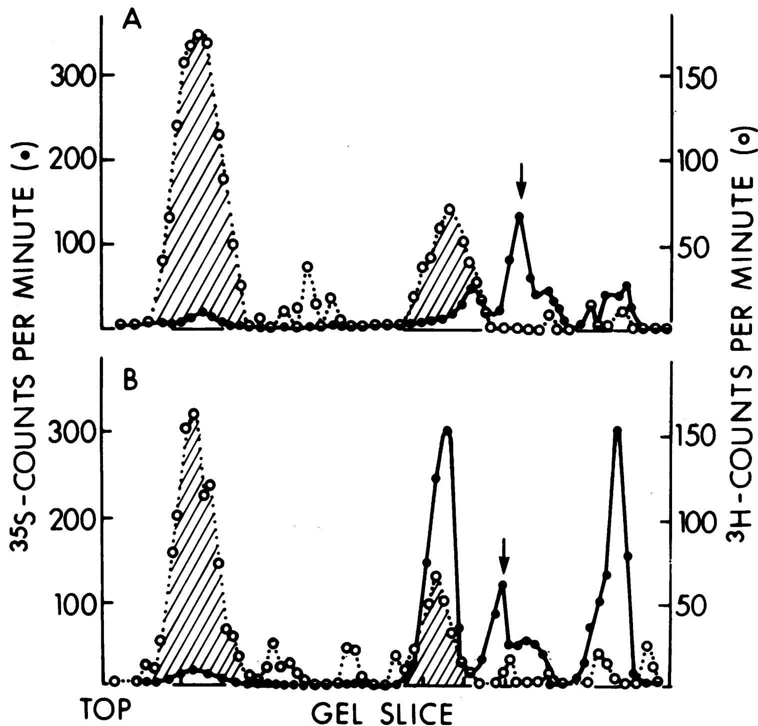

FIG. 2. *Analysis of RSV proteins by electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel. Samples of* ^{3}H*- and* ^{35}S*-labeled RSV from the peak region of the sucrose gradient (Fig. 1A) and from the radioimmune precipitates obtained from samples of that peak were electrophoresed according to procedures described by Schlesinger et al. (18). Refer to text for additional experimental details. (A) Sample from the radioimmune precipitate; (B) sample not precipitated. The arrow refers to p19 of RSV.*

prising, however, is the retention in this precipitate of the RSV p19 component. The amount of p19 (arrow, Fig. 2) relative to gp37 and gp85 is the same for both precipitated and nonprecipitated material. Hung et al. (10) and Duesberg et al. (4) also found a protein of about the size of p19 when the RSV envelope was isolated by treatment of virus with detergent. The p19 protein has not been specifically localized to either the core or the envelope fractions of RSV (3), and p19 is also reported to contain type-specific regions (2, 8). The p19 may be an integral part of the envelope structure of RSV, serving to anchor the glycoproteins in the membrane of the virion. There is also the possibility that the retention of p19 in the immune precipitate is the result of a nonspecific trapping of this protein during the Nonidet P-40 washings of the precipitate.

The experiments reported here show that an infectious RSV-antibody complex can be formed by the binding to virions of antibodies directed against regions of the major envelope protein. We have found recently a similar situation for the formation in vitro of an infectious virus-antibody complex between Sindbis virus and antibodies raised against one of the isolated glycoproteins of the virion (M. Schlesinger et al., unpublished data). Antibodies directed against unique determinants of viral envelope proteins may prove to be part of the naturally occurring infectious complexes reported for a variety of animals chronically infected with virus (12, 14). Oldstone et al. (13) have recovered antibodies from complexes deposited in the kidneys of AKR leukemic mice and have shown that these antibodies react with several components of the murine leukemia virus. Infectious complexes have been isolated from kidneys of mice given Moloney virus (9), and it will be of interest to determine if the antibodies in these complexes have specificity for the glycoprotein of the murine leukemia virus.

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