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Specific Antigenic Relationships Between the RNA-Dependent DNA Polymerases of Avian Reticuloendotheliosis Viruses and Mammalian Type C Retroviruses

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Immunoglobulin G directed against the DNA polymerase of Rauscher murine leukemia virus (R-MuLV) could bind to ^{125}I-labeled DNA polymerase of spleen necrosis virus (SNV), a member of the reticuloendotheliosis virus (REV) species. Competition radioimmunoassays showed the specificity of this cross-reaction. The antigenic determinants common to SNV and R-MuLV DNA polymerases were shared completely by the DNA polymerases of Gross MuLV, Moloney MuLV, RD-114 virus, REV-T, and duck infectious anemia virus. Baboon endogenous virus and chicken syncytial virus competed partially for antibodies directed against the common antigenic determinants of SNV and R-MuLV DNA polymerases. DNA polymerases of avain leukosis viruses, pheasant viruses, and mammalian type B and D retroviruses and particles with RNA-dependent DNA polymerase activity from the allantoic fluid of normal chicken eggs and from the medium of a goose cell culture did not compete for the antibodies directed against the common antigenic determinants of SNV and R-MuLV DNA polymerases. We also present data about a factor in normal mammalian immunoglobulin G that specifically inhibits the DNA polymerases of REV and mammalian type C retrovirus DNA polymerases.

Reticuloendotheliosis viruses (REV) are a species of avian retroviruses (20, 21). They are distinct from avian leukosis-sarcoma viruses (ALV) and pheasant viruses (PV) in their protein structure and RNA nucleotide sequence (7, 8, 10, 14). Within the REV species there is strong homology of the genome (10, 11), which is reflected in homology of the structural proteins (2, 15).

The REV are exogenous avian retroviruses. The DNA of the uninfected avian cells tested hybridizes at most to 10% of the RNA of REV (11). Thus, the origin of REV is uncertain. Morphological studies of the virions (19, 23) and characterization of the REV DNA polymerase, a 70,000- to 80,000-dalton polypeptide (18, 19), indicated a similarity to mammalian type C retroviruses (19). Although an initial immunological characterization of the active site of REV DNA polymerase showed the ALV and REV DNA polymerases are different from each other (16), the ability of REV DNA polymerase to absorb antibody directed against the active site of ALV DNA polymerase was taken as an indication of serological relationship between the DNA polymerases of the two species (17). This relationship was interpreted as indicating an evolutionary relationship between ALV and REV and a common origin of both viruses from normal cells.

Recent observations have, however, shown that there are amino acid sequence homologies and common immunological determinants between the major core proteins of REV and mammalian type C retroviruses (2, 6, 9).

The availability of a radioimmunoassay for spleen necrosis virus (SNV) DNA polymerase (5) prompted us to reinvestigate the relationship of REV DNA polymerase to the DNA polymerases of other retroviruses, including mammalian retroviruses. In a preceding paper (5), we showed that REV DNA polymerase is distinct from ALV and PV DNA polymerases. There was no indication of a relationship between REV and ALV or PV DNA polymerases when radioimmunological methods were used. (There was little variation detected between the DNA polymerases of the four members of the REV species.)

Here we report the existence of antigenic determinants common to REV and mammalian type C retrovirus DNA polymerases. We also present data on an inhibitory factor in normal mammalian immunoglobuin G (IgG) specific for REV and mammalian type C retrovirus DNA polymerases.

MATERIALS AND METHODS

Viruses. Viruses used in this study have been de-

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scribed previously (5). Particles containing RNA-dependent DNA polymerase activity were isolated from embryonated chicken eggs (SPAFAS, gs^- chf^-) or from the supernatant medium of cells from a goose embryo, as previously described (3, 4).

IgG. Preparation of antisera to RNA-dependent DNA polymerases of retroviruses has been described (5). IgG was purified from serum by chromatography on DEAE-Affi-Gel Blue (Bio-Rad Laboratories). Sera were dialyzed against 20 mM Tris-hydrochloride (pH 7.8)-28 mM NaCl and passed through the resin (1 ml of serum/5 ml of resin). The unbound material was collected and brought to 50% ammonium sulfate, and the precipitate was collected by low-speed centrifugation. The pellet was resuspended and dialyzed against 20 mM Tris-hydrochloride (pH 7.8)-20 mM NaCl and stored at −70°C.

IgG inhibition test. The IgG inhibition test is described in the legend of Fig. 1.

Labeling of DNA polymerases with ^{125}I. The iodinated DNA polymerases have been described (5). Purified DNA polymerases were iodinated by the chloramine-T method. After separation from free iodide, the labeled material was centrifuged in a glycerol gradient. Full-size, labeled DNA polymerases were recovered and shown to be homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Binding assay. Direct assay for binding of IgG to SNV DNA polymerase is described in the legend to Fig. 2.

Immunoprecipitation. Immunoprecipitation by the double-antibody technique was carried out in a reaction volume of 25 μl, using purified IgG. Thus, high concentrations of protease-free IgG could be used. The exact conditions are described in the legend to Fig. 4.

Second antibodies. Rabbit antibody to goat IgG and goat antibody to rabbit IgG were obtained from Calbiochem-Behring. The lyophilized antibodies were dissolved in radioimmunoassay buffer (50 mM Tris-hydrochloride [pH 7.8]-200 mM KCl-10% glycerol-0.4% Triton X-100-5 mg of bovine serum albumin per ml [A grade]) before use. Competition immunoassay is described in the legend to Fig. 5.

Protein determination. The method described by Lowry et al. (13) was used.

RESULTS

Inhibition of REV DNA polymerase activity and mammalian type C retrovirus DNA polymerase activity by normal sera and antisera. In an initial experiment, we tested for a possible relationship between REV DNA polymerase and mammalian retrovirus DNA polymerases, using the classical IgG inhibition test. Disrupted SNV, avian myeloblastosis virus (AMV), and mammalian type B, C, and D retroviruses were preincubated with rabbit IgG directed against SNV DNA polymerase, goat IgG directed against Rauscher murine leukemia virus (R-MuLV) DNA polymerase, or the corresponding preimmune IgG, and then the residual DNA polymerase activities were determined. SNV DNA polymerase was very effectively inhibited by IgG against SNV DNA polymerase (Fig. 1A). Less than 0.4 μg of IgG was necessary to inhibit enzyme activity to 20% residual activity; 50- to 100-fold-higher concentrations of IgG caused comparable inhibition of the activity of DNA polymerases of baboon endogenous virus (BEV), RD-114 virus, or R-MuLV. These DNA polymerases were completely inactivated by 100 μg of IgG per assay. The activities of DNA polymerases of AMV, murine mammary tumor virus (MMTV; type B retrovirus), or Mason-Pfizer monkey virus (MPMV; type D retrovirus) were not inhibited significantly under the test conditions.

In a complementary experiment, goat IgG against the DNA polymerase of R-MuLV was shown to inactivate R-MuLV DNA polymerase efficiently and to have a considerable inhibitory effect on the activity of SNV DNA polymerase and the DNA polymerases of BEV and RD-114 virus (Fig. 1B). Only three- to fourfold-higher concentrations of IgG were required for comparable inactivation of R-MuLV and the other DNA polymerases. Again there was no inhibitory effect on the DNA polymerases of AMV, MMTV, and MPMV.

Parallel tests of the respective normal IgG's showed, however, that even in the absence of active immunization, rabbit and goat IgG contained inhibitory activity specific for REV and mammalian type C virus DNA polymerases. This inhibition was less pronounced with the normal rabbit IgG (Fig. 1C), but very clear with the normal goat IgG (Fig. 1D). The normal goat IgG caused 80 to 90% inhibition of the activity of some DNA polymerases. Similar results were also seen with normal rat IgG (Fig. 1E), normal dog serum (data not shown), and normal human serum (data not shown). In each case the inhibitory effect was specific for REV and mammalian type C virus DNA polymerases. ALV, MMTV, and MPMV DNA polymerases were not inhibited significantly. The specificity of the inhibitory effect for certain DNA polymerases demonstrated that the inhibition was not the result of a nonspecific interference of the IgG preparation with the DNA polymerase assay.

The inhibition experiments were all carried out with IgG's purified by passage through DEAE-Affi-Gel Blue, a resin that specifically binds serum proteases. Later (Fig. 2) it will be shown directly that the inhibitory effect was not the result of gross proteolytic degradation.

Two conclusions can be drawn from the IgG inhibition studies: (i) normal IgG contains inhibitory factors specific for REV and mammalian type C virus DNA polymerases, and (ii) demonstration of a cross-reaction between REV and

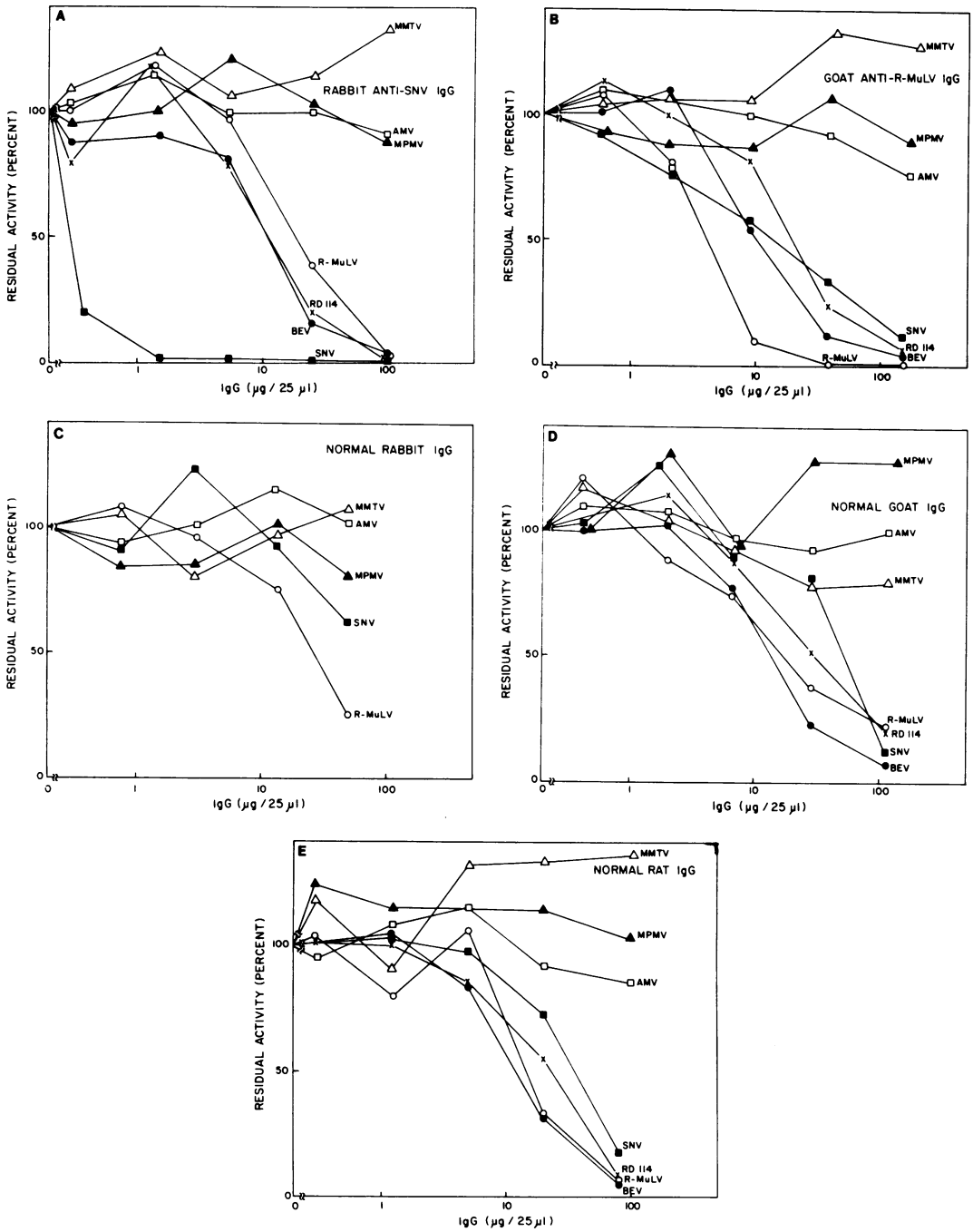

FIG. 1. *IgG inhibition tests of RNA-dependent DNA polymerases. Purified IgG was serially diluted in 20 mM Tris-hydrochloride (pH 7.8)–20 mM KCl and preincubated for 30 min at room temperature in a total volume of 25 μl with constant amounts of disrupted viruses (2 to 5 μg/assay) in the presence of 150 mM KCl, 20 mM Tris-hydrochloride (pH 8), and 0.2% Triton X-100. The assays were brought to DNA polymerase assay conditions (4) by using activated calf thymus DNA as a template·primer and were further incubated for 40 min at 37°C. The acid-insoluble radioactivity was determined, and the residual activity relative to assays without IgG (= 100%) was calculated. Viruses used were: SNV (■), R-MuLV (○), BEV (●), RD-114 (×), AMV (□), MMTV (△), and MPMV (▲). (A) Rabbit IgG against SNV DNA polymerase; (B) goat IgG to R-MuLV DNA polymerase; (C) normal rabbit IgG; (D) normal goat IgG; (E) normal rat IgG.*

mammalian type C virus DNA polymerases by IgG inhibition tests is, therefore, inconclusive.

Binding of antibody to ^{125}I-labeled SNV DNA polymerase. To examine further the reaction between SNV DNA polymerase and the IgG's mentioned above, we established a direct binding assay. Various IgG's, at a concentration shown to inhibit SNV DNA polymerase to 10 to 20% residual activity in an IgG inhibition test, were preincubated with ^{125}I-labeled SNV DNA polymerase under conditions identical to those in an IgG inhibition test. Then the samples were subjected to gradient ultracentrifugation to check for the formation of complexes sedimenting faster than DNA polymerase. None of the normal IgG's changed the sedimentation coefficient of SNV DNA polymerase (Fig. 2). This result demonstrates that (i) there is no stable binding between the normal IgG's tested and labeled SNV DNA polymerase, and (ii) since SNV DNA polymerase remains intact as judged by its sedimentation rate, the inhibition of SNV DNA polymerase by normal IgG's is not the result of gross proteolytic degradation.

In contrast, IgG's directed against SNV DNA polymerase or R-MuLV DNA polymerase formed a stable complex with SNV DNA polymerase (Fig. 2 and 3). The complex between anti-R-MuLV IgG and ^{125}I-labeled SNV DNA polymerase sedimented faster than IgG alone and with the same rate as a complex between 0.4 μg of anti-SNV IgG and ^{125}I-labeled SNV DNA polymerase; 0.4 to 1 μg of anti-SNV IgG had the same effect as 150 μg of anti-R-MuLV IgG. When higher concentrations of anti-SNV IgG were used, faster-sedimenting complexes were seen (Fig. 2, 1.6 μg of anti-SNV IgG).

Immunoprecipitation of ^{125}I-labeled SNV DNA polymerase. The data obtained from the direct binding assay (Fig. 2 and 3) indicated that IgG against R-MuLV DNA polymerase contained antibodies reacting with SNV DNA polymerase. To prove the specificity of this cross-reaction, a double-antibody immunoprecipitation with ^{125}I-labeled SNV DNA polymerase and anti-R-MuLV DNA polymerase IgG was performed.

IgG against R-MuLV DNA polymerase, purified by DEAE-Affi-Gel Blue chromatography, was tested with ^{125}I-labeled SNV DNA polymerase (Table 1). Both goat antibody to R-MuLV and rabbit antibody to goat IgG were required in order to demonstrate by low-speed centrifugation an immunocomplex containing ^{125}I-labeled SNV DNA polymerase. If goat antibody to R-MuLV DNA polymerase was replaced by normal goat IgG or goat IgG against AMV DNA polymerase, ^{125}I-labeled SNV DNA polymerase was not precipitated. The binding of ^{125}I-labeled

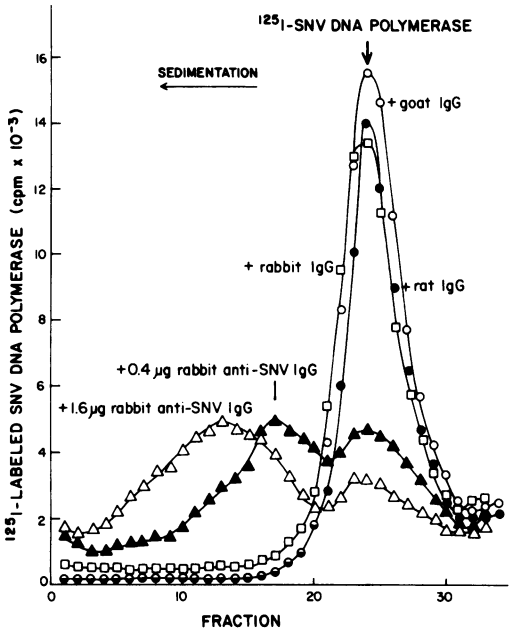

FIG. 2. *Assay for binding of IgG to SNV DNA polymerase. ^{125}I-labeled SNV DNA polymerase (100,000 cpm) was preincubated with various purified IgG's in the presence of 150 mM KCl, 20 mM Tris-hydrochloride (pH 8), and 1 mg of bovine serum albumin per ml in a total volume of 25 μl for 30 min at room temperature. The samples were diluted with 150 μl of radioimmunoassay buffer and loaded on 10 to 30% glycerol gradients (50 mM Tris-hydrochloride [pH 7.8]-150 mM KCl-0.4% Triton X-100). Centrifugation (rotor SW-50.1, Beckman) was at 40,000 rpm for 22 h at 4°C. The gradients were fractionated from the bottom, and the radioactivity per fraction was determined. Symbols: ○, 110 μg of normal goat IgG; ●, 80 μg of normal rat IgG; □, 100 μg of normal rabbit IgG; ▲, 0.4 μg of rabbit IgG to SNV DNA polymerase; △, 1.6 μg of rabbit IgG to SNV DNA polymerase. The position of ^{125}I-SNV DNA polymerase incubated in the absence of IgG is indicated by an arrow. The position of ^{125}I-SNV DNA polymerase incubated with goat antibody to R-MuLV DNA polymerase is shown in Fig. 3.*

SNV DNA polymerase by antibody to R-MuLV DNA polymerase was strictly dependent on incubation with first and second antibodies. Incubation for 18 h at 4°C rather than 30 min at room temperature, as in this experiment, enhanced the binding by 20% (data not shown). Goat anti-rabbit IgG did not bind to the complex between labeled SNV DNA polymerase and goat anti-R-MuLV DNA polymerase IgG. Furthermore, this complex was not trapped in an immunocomplex between normal rabbit serum and goat anti-rabbit IgG. Finally, the reaction was specific for ^{125}I-labeled SNV DNA polymerase, since ^{125}I-labeled AMV DNA polymerase could

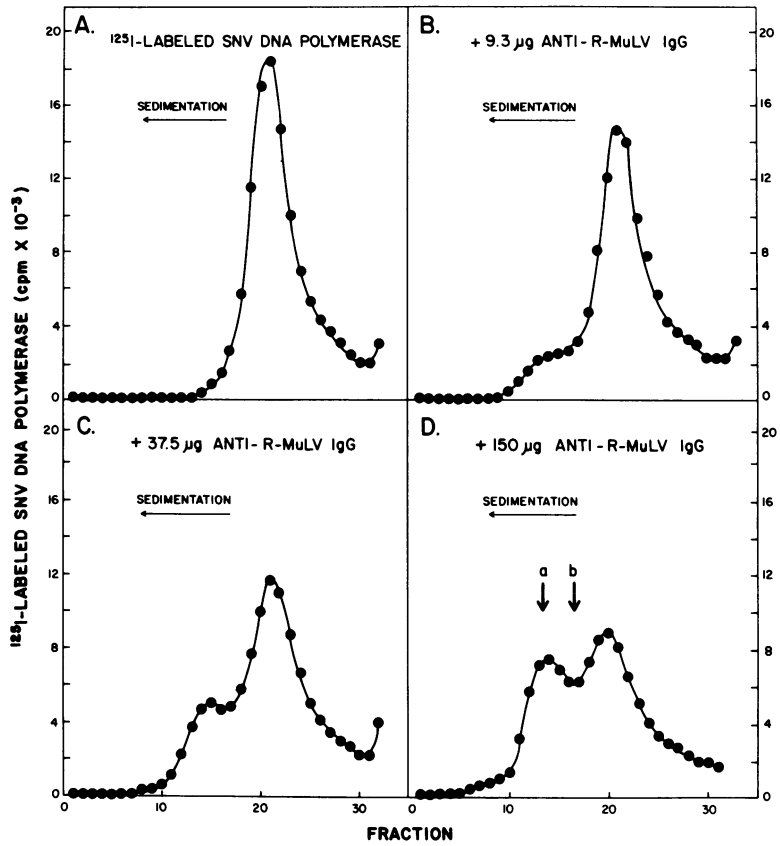

FIG. 3. *Binding of IgG against R-MuLV DNA polymerase to ^{125}I-labeled SNV DNA polymerase. The experiment was performed as described in the legend of Fig. 2. ^{125}I-labeled SNV DNA polymerase was incubated without IgG (A) or in the presence of increasing amounts of goat IgG against R-MuLV DNA polymerase: 9.3 μg (B), 37.5 μg (C), 150 μg (D). Centrifugation was at 45,000 rpm for 20 h at 4°C. (D) a, Position of ^{125}I-labeled SNV DNA polymerase preincubated with 0.4 μg of rabbit IgG against SNV DNA polymerase in a parallel gradient; b, position of free IgG sedimenting in a parallel gradient.*

not be bound significantly by anti-R-MuLV antibody under complete reaction conditions.

These data show that the precipitation of ^{125}I-labeled SNV DNA polymerase by IgG against R-MuLV DNA polymerase by using the double-antibody technique was the result of specific immune reactions. This experiment also defines the background values observed under our conditions: 500-cpm absorption of ^{125}I-labeled DNA polymerase to the tube in the absence of an immunoprecipitate, and 1,500-cpm nonspecific trapping of DNA polymerase in a precipitate.

The reaction conditions described in Table 1 were used to study the immunoprecipitation of ^{125}I-labeled SNV DNA polymerase by IgG's against various viral DNA polymerases (Fig. 4). Six micrograms of goat antibody to R-MuLV DNA polymerase was required for 50% precipitation. Much less (0.01 μg) anti-SNV IgG was required for the same effect (Fig. 4A). IgG against the DNA polymerases of BEV and AMV showed 10% precipitation above background. The significance of this effect is unknown. Preimmune IgG's, as well as IgG's against the DNA polymerases of Rous-associated virus-61 (RAV-61), Rous sarcoma virus-RAV-0 (RSV-RAV-0), and Amherst pheasant virus (APV), did not cause significant precipitation of SNV DNA polymerase. Control experiments showed that the IgG's against ALV and PV DNA polymerases were able to precipitate ^{125}I-labeled AMV DNA polymerase very well (Fig. 4B). In contrast, IgG against SNV DNA polymerase could not precipitate AMV DNA polymerase.

These data indicated a specific cross-reaction between antibody to R-MuLV DNA polymerase and SNV DNA polymerase.

Competition radioimmunoassays. To test

TABLE 1. *Precipitation of SNV DNA polymerase by antibody to R-MuLV DNA polymerase*[a]

^{125}I-DNA polymerase	First antibody	First incubation	Second antibody	Visible precipitate formed	cpm precipitated
SNV		+		−	450
SNV		+	RAG	−	600
SNV	Goat anti-R-MuLV	+		−	460
SNV	Goat anti-R-MuLV	+	RAG	+	12,350
SNV	Normal goat	+	RAG	+	1,930
SNV	Goat anti-AMV	+	RAG	+	1,750
SNV	Goat anti-R-MuLV	−	RAG	+	1,300
SNV	Goat anti-R-MuLV	+	GAR	−	500
SNV	Goat anti-R-MuLV	+	GAR[b]	+	1,050
AMV	Goat anti-R-MuLV	+	RAG	+	1,200
AMV	Goat anti-AMV	+	RAG	+	28,300

[a] ^{125}I-labeled SNV DNA polymerase (24,000 cpm) or ^{125}I-labeled AMV DNA polymerase (30,000 cpm) was preincubated with 35 μg of the indicated IgG's or without IgG (first incubation). The reaction was carried out in the presence of 150 mM KCl, 20 mM Tris-hydrochloride (pH 7.8), and 1 mg of bovine serum albumin per ml in a volume of 25 μl. Incubation was for 30 min at room temperature. Assay 7, containing goat anti-R-MuLV, was not preincubated. Then 150 μl of rabbit antibody to goat IgG (RAG) or goat antibody to rabbit IgG (GAR) or no second antibody was added. Incubation was at 4°C for 4 h. The immunoprecipitates were collected by low-speed centrifugation. Goat anti-R-MuLV and goat anti-AMV were purified IgG's directed against the DNA polymerases of R-MuLV and AMV, respectively.

[b] 5 μl of normal rabbit serum was present to allow formation of a precipitate.

the specificity of the cross-reaction shown in Fig. 4A, disrupted viruses and particles with reverse transcriptase activity from uninfected cells were tested for their ability to compete for binding of SNV DNA polymerase to antibody against R-MuLV DNA polymerase (Fig. 5A). Gross MuLV, Moloney MuLV (Mo-MuLV), and R-MuLV competed with the same characteristic slope and to the same extent as SNV. The slope of the competition curve by RD-114 virus was different, but the final competition was essentially complete. BEV could only compete about 50% and then reached a plateau. MMTV, MPMV, AMV, APV, chicken particles with RNA-dependent DNA polymerase activity, and goose particles with RNA-dependent DNA polymerase activity did not compete at all. Within the REV species, SNV, REV-T, and duck infectious anemia virus (DIAV) competed identically, whereas chicken syncytial virus (CSV) competed incompletely and with a much shallower curve. These data show that there is a set of antigenic determinants common to REV and mammalian type C viruses, indicating that these two groups of viruses should be classified in one genus of retroviruses. Some members of the genus only share part of the set of antigenic determinants. Members of other viral species, like mammalian type B and D retroviruses, ALV, and PV, as well as particles with reverse transcriptase activity from normal chicken and goose cells, do not possess these antigenic determinants.

DISCUSSION

Inhibitors of REV and mammalian type C retrovirus DNA polymerases in normal IgG. The data obtained by IgG inhibition tests in this study show that IgG purified from the sera of normal animals contains an inhibitory factor for the DNA polymerases of REV and mammalian type C retroviruses. The inhibition by this factor is different from the nonspecific inhibition commonly observed when relatively high concentrations of serum rather than IgG are used, since the nonspecific inhibition does not distinguish between the DNA polymerases of retroviruses of different species. In contrast, the inhibitory factor demonstrated in purified IgG of rabbits, rats, goats (Fig. 1), dogs, and humans (data not shown) specifically inhibits mammalian type C retrovirus and REV DNA polymerases, but has no effect on the DNA polymerases of mammalian type B and D retroviruses and ALV. It is not clear whether there is one type of inhibitory factor in normal IgG reacting with DNA polymerases of both the REV species and mammalian type C retroviruses, or there are different independent populations of factors. Absorption studies to clarify this question were unsuccessful (data not shown). This failure might be explained by the finding that no stable binding of inhibitory factors to SNV DNA polymerase could be demonstrated (Fig. 2).

It is obvious that antisera made against a specific retrovirus DNA polymerase will also

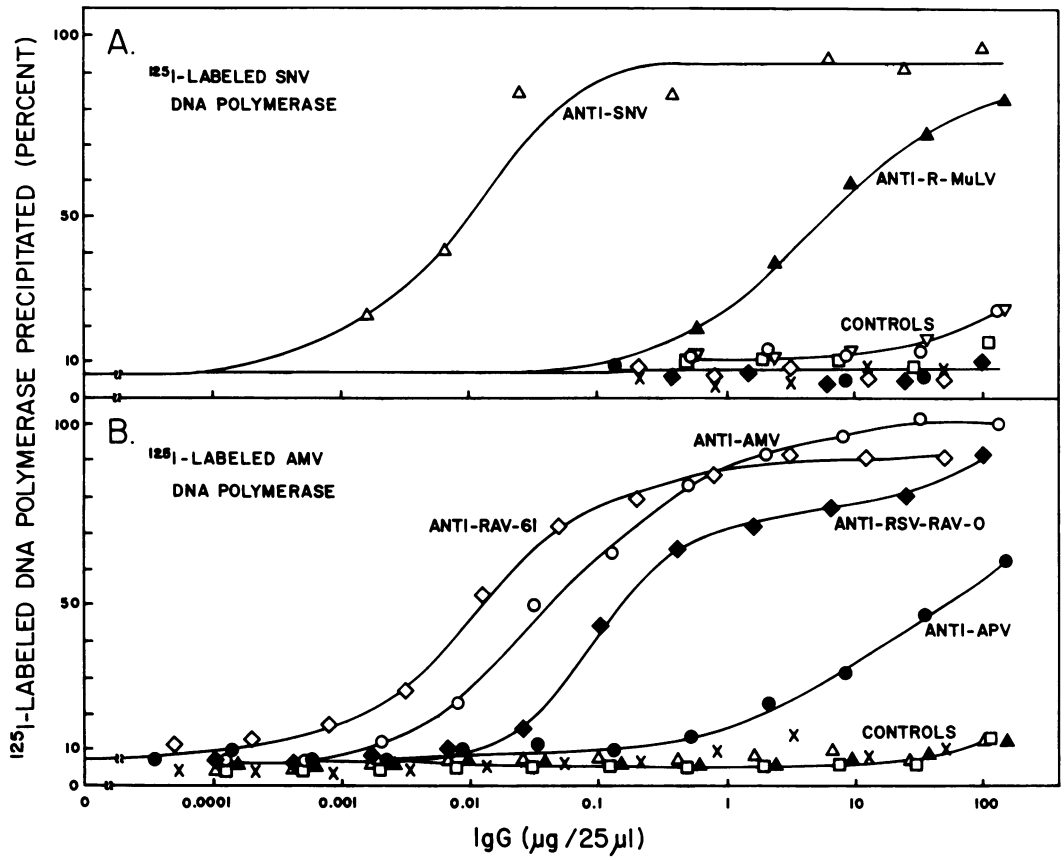

FIG. 4. *Radioimmunoprecipitation of SNV DNA polymerase and AMV DNA polymerase. Serial dilutions of purified IgG's were incubated with ^{125}I-labeled SNV DNA polymerase (A) or ^{125}I-labeled AMV DNA polymerase (B) in a volume of 25 μl for 18 h at 4°C in the presence of 150 mM KCl, 20 mM Tris-hydrochloride (pH 8), and 1 mg of bovine serum albumin per ml. Normal serum and second antibody were added in amounts determined to be optimal for immunoprecipitation. In the case of assays containing rabbit IgG, the total amount of IgG present was brought to 50 μg; 150 μl of goat antibody to rabbit gamma globulin were added. Assays with higher IgG amounts received 300 to 400 μl of second antibody. In the case of goat IgG, assays were brought to 25 μg of IgG, and 150 μl of rabbit antibody to goat gamma globulin was added. Assays with higher IgG amounts received 300 μl of second antibody. The assays were incubated for another 4 to 5 h at 4°C, and the immunoprecipitates were collected. Symbols: Rabbit IgG to* (△) *SNV DNA polymerase,* (◇) *RAV-61 DNA polymerase,* (◆) *RSV-RAV-0 DNA polymerase, and* (●) *APV DNA polymerase;* (×) *normal rabbit IgG; goat IgG to* (▲) *R-MuLV DNA polymerase,* (▽) *BEV DNA polymerase, and* (○) *AMV DNA polymerase;* (□) *normal goat IgG.*

contain the natural inhibitory factor(s) for REV and mammalian type C retrovirus DNA polymerases. Therefore, findings of cross-reactions between different retrovirus DNA polymerases in simple IgG inhibition tests (using immune IgG against a particular DNA polymerase) do not necessarily indicate relationships, since the effects of the immune IgG and the natural inhibitory factor(s) cannot be differentiated by this method.

Since the inhibitory factor(s) is found in purified IgG, sediments at 7S (data not shown), and shows specificity for certain DNA polymerases, one might assume that it is an antibody molecule. However, it would have to be an antibody with low affinity, since no stable binding to SNV DNA polymerase could be demonstrated in direct binding assays.

In agreement with the idea that the natural inhibitory factor is an antibody are the following findings: (i) sera from germfree animals show no (in the case of dog serum) or strongly reduced (less than 10% of normal sera in the case of rat serum) titers of inhibitory factor (data not shown), and (ii) sera from newborn rats are negative (data not shown).

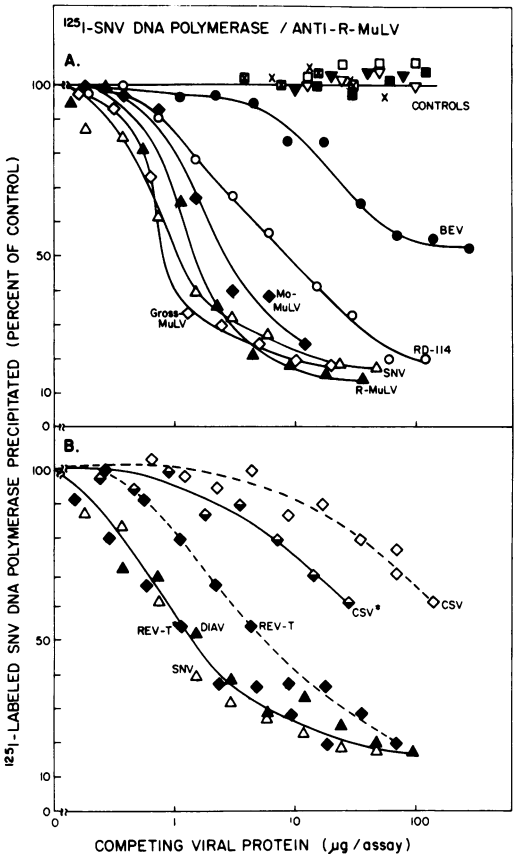

FIG. 5. *Competition radioimmunoassay. Serial twofold dilutions of viruses or particles were incubated with 37.5 μg of goat antibody to R-MuLV DNA polymerase in a total volume of 40 μl in the presence of 190 mM KCl, 0.2% Triton X-100, 3 mg of bovine serum albumin per ml, and 25 mM Tris-hydrochloride (pH 7.8) for 1 h at 37°C. After cooling, 10 μl of rabbit antibody to goat gamma globulin and 150 μl radioimmunoassay buffer were added, and the immunoprecipitates were collected after 4 h. (A) Competition by SNV, mammalian type C viruses and controls (AMV, ▽; APV, ▼; chicken particles with RNA-dependent DNA polymerase activity, ⊠; goose particles with RNA-dependent DNA polymerase activity, ×; MMTV, ■; MPMV, □). (B) Competition by REV. Since the CSV and REV-T used in this study were previously shown to contain less DNA polymerase per mg of viral protein than SNV or DIAV (5), curves normalized for an equivalent DNA polymerase content relative to SNV are included (CSV*, REV-T*).*

Another explanation for a specific inhibitory activity might be the action of a specific protease. Analysis of ^{125}I-labeled SNV DNA polymerase on glycerol gradients after incubation with IgG showed, however, that the molecules remained intact. This result excludes gross proteolytic degradation as a cause of the inhibition.

Several other groups have observed antibodies against retrovirus proteins in normal human sera (1, 12, 22). The origin and significance of naturally occurring antibodies against retroviral proteins remains unclear. Their frequent occurrence has been interpreted to indicate a relatively high degree of spread of retroviruses through populations.

Our findings might also be interpreted along those lines. However, we cannot yet state that the inhibitory factor described here is a naturally occurring antibody. We stress the practical aspect of the finding: Simple IgG inhibition tests of DNA polymerases of certain viral species may lead to false interpretations, especially when high concentrations of IgG are used, as a result of the presence of naturally occurring inhibitory factors.

Specific relationships between REV and mammalian type C retrovirus DNA polymerases. Whereas both normal and immune IgG's used in the experiment described in Fig. 1 could inhibit SNV DNA polymerase, only immune IgG's against the DNA polymerases of SNV or R-MuLV were able to form a sedimentable complex with iodinated SNV DNA polymerase. The formation of a 10S complex between SNV DNA polymerase and IgG against the DNA polymerase of R-MuLV was dependent on the concentration of IgG.

The amount of IgG against R-MuLV DNA polymerase necessary for binding 50% of labeled SNV DNA polymerase was much higher than the amount of IgG against SNV DNA polymerase required for the same degree of binding. This result indicates that only a subpopulation of IgG molecules in the anti-R-MuLV IgG cross-reacts with SNV DNA polymerase.

Based on the conditions of the direct binding assay, double-antibody immunoprecipitations were performed. The binding of IgG against R-MuLV DNA polymerase to SNV DNA polymerase was shown to have the characteristics of a specific immune reaction. ^{125}I-labeled SNV DNA polymerase could only be precipitated by homologous anti-SNV or anti-R-MuLV IgG, not by IgG directed against the DNA polymerases of ALV or PV. The failure of anti-BEV IgG to bind SNV DNA polymerase was probably the result of the low titer of this IgG (compared with anti-R-MuLV IgG, it shows 1/50 of neutralizing capacity in an homologous IgG inhibition test).

Our data show that there is a specific immunological cross-reaction between the DNA polymerases of REV and mammalian type C retroviruses. The antigenic determinants recognized by antiserum to R-MuLV DNA polymerase on SNV DNA polymerase are completely

shared by SNV, REV-T, DIAV, Gross MuLV, R-MuLV, Mo-MuLV, and RD-114 virus. BEV and CSV share part of the determinants. ALV, PV, mammalian type B and type D retrovirus DNA polymerases, as well as the RNA-dependent DNA polymerases from particles isolated from normal chicken or goose cells do not share the determinants common to REV and mammalian type C viruses. These data define REV and mammalian type C virus DNA polymerases as one genus of DNA polymerases.

The finding that SNV, DIAV, REV-T, and MuLV DNA polymerases compete to the same extent and with the same characteristic curve for antibody against the common determinants parallels the finding for common determinants of the p30 proteins of these viruses (2). The same is true for the partial competition of BEV DNA polymerase. In contrast to the results with p30 proteins, we find that RD-114 virus DNA polymerase can compete completely (although with a somewhat different slope) and that CSV cannot. We have previously shown that CSV DNA polymerase, except for a minor type-specific difference, can compete for all antibodies directed against SNV DNA polymerase (5). CSV, was therefore classified as a member of the REV species, and the inability of CSV DNA polymerase to compete for the antibodies directed against the common determinants of REV and mammalian type C retrovirus DNA polymerases is interpreted as a loss of antigenic determinants rather than as a different evolutionary history. This interpretation agrees with the finding that CSV p30 shares all the determinants common to REV-T p30 and mammalian type C retrovirus p30 (2).

The modified radioimmunoassay used in this study (use of protease-free IgG in a small reaction volume) showed the advantage of a higher sensitivity for detecting immunological cross-reactions than the standard assay previously used. Reinvestigation of the cross-reactions between avian retrovirus DNA polymerases with the modified test confirmed our previous findings (5): ALV and PV DNA polymerases are grossly different from each other but share some common determinants. No relationship between these two viral species and REV could be demonstrated in radioimmunoassays. The results of this paper and the fact that radioimmunological techniques failed to demonstrate a significant relationship between REV and ALV or PV indicate that the ability of REV DNA polymerase to absorb antibody against the active site of ALV DNA polymerase (17) does not suggest a common evolutionary origin of ALV and REV DNA polymerases, but rather might reflect a common feature, reached in parallel evolution. This feature is not outstanding enough to allow cross-reaction in immunoprecipitation reactions or competition radioimmunoassay. In contrast, we could show that REV and mammalian type C virus DNA polymerases cross-react specifically and to a degree comparable to that found for ALV and PV.

The common determinants between REV and mammalian type C retroviruses are only a small subpopulation of antigenic determinants of SNV DNA polymerase, as indicated by the finding that none of the mammalian type C retrovirus DNA polymerases could compete significantly in a homologous radioimmunoassay (^{125}I-labeled SNV DNA polymerase/antibody to SNV DNA polymerase) (5). This finding indicates that REV and mammalian type C retrovirus DNA polymerases are grossly different from each other, but share some conserved antigenic determinants. This finding is also a formal argument against a trivial explanation of the results demonstrated here which assumes that the mammalian retroviruses used were contaminated with REV.

Our data on the relationship of REV and mammalian type C retrovirus DNA polymerases support the idea that REV originated from a mammalian type C retrovirus that infected birds. Origin of REV from mammalian type C retroviruses has been proposed several times in the past, based on morphological (19, 23), biochemical (9, 19), and immunological (2, 6) findings. Our data are a direct proof for the existence of an evolutionary relationship of the DNA polymerases of REV and mammalian type C viruses, originally proposed by Moelling et al. (19).

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