

Natural human antibodies reactive with primate type-C viral antigens*

(human sera/oncornavirus proteins)

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ABSTRACT A survey of human sera from healthy individuals revealed the presence of naturally occurring antibodies that react in radioimmunoprecipitation assays with proteins of mammalian type-C viruses. Of 39 sera tested, 100% showed reactivity against baboon endogenous virus, whereas only 49% showed reactivity against simian sarcoma-associated virus. Polyacrylamide gel electrophoresis of immune precipitates revealed one to three bands that comigrate with the virus structural proteins. There were low, but detectable, levels of antibody to the major internal protein of murine leukemia virus, but no activity against the structural proteins of avian sarcoma virus.

A number of reports describe the isolation of infectious, replicating type-C viruses from human tissues (1-5). There have also been reports on the presence of antigens in tissues of normal individuals as well as of patients with neoplastic or autoimmune diseases that react with antisera specific to structural proteins of mammalian type-C viruses (6-9).

One approach to investigating the natural history of type-C infection in people is to test sera from healthy donors and from patients with various diseases for the presence of antibodies against viruses. Most populations of chickens, mice, cats, and gibbons possess natural antibodies against their respective horizontally or vertically transmitted oncornavirus strains (10-14). Furthermore, several laboratories (15-18) have reported the presence of antibodies in normal human sera and in the sera from patients with leukemia that react with proteins of primate type-C viruses. On the other hand, two reports document a failure to find such antibodies (19, 20). The recent isolates (1-5) of replicating virus from human tissues proved to be closely related to the simian sarcoma-gibbon ape lymphoma virus group or to the endogenous baboon viruses. We have therefore used the simian sarcoma-associated helper virus (SSAV) (21) and the M7 strain of baboon endogenous virus (BEV) (22) to survey human sera for specific antibodies. Also included is a virus (HL23V-1) originally isolated from cultured peripheral leukocytes of a patient with acute myelogenous leukemia (23). HL23V-1 was shown to comprise a mixture of two viruses, one closely related to SSAV, the other to BEV (5, 24, 25). Our data indicate that sera of many normal humans possess precipitating activity for the small structural virus proteins (p28, p15, or p15E) as well as for the large envelope glycoprotein (gp70) of SSAV and BEV, and for the p30 of Moloney murine leukemia virus; avian oncornavirus proteins could not be precipitated.

Abbreviations: BEV, baboon endogenous virus; SSAV, simian sarcoma-associated virus; GALV, gibbon age lymphoma virus; MuLV, murine leukemia virus; gp70, p28, or p15, nomenclature for structural proteins of RNA tumor viruses (see August *et al.*, ref. 38); NaDodSO₄, sodium dodecyl sulfate.

* We dedicate this paper to Dr. Ian Macpherson.

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This report deals with the immunological specificities of the natural human antibodies.

MATERIALS AND METHODS

Cells and Viruses. The HL23V-1 virus complex (23) was propagated in nonproducer NRK cells transformed by Kirsten strain murine sarcoma virus (KNRK) (5). The KNRK isolate contains the SSAV-like virus only; it does not contain detectable virus proteins of the Kirsten sarcoma virus. Moloney strain of murine leukemia virus (MuLV) was grown in NIH-3T3 cells. BEV was propagated in dog thymus cells and SSAV in KNRK cells (kindly provided by Dr. Mayyasi, Pfizer Inc.). Chicken embryo fibroblasts (Lohman, Cuxhaven, W. Germany) were used for the production of Prague strain avian sarcoma virus. Cells were grown in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum and antibiotics. Virus was pelleted from supernatant media and partially purified in 25-55% sucrose gradients (150,000 × *g*, 80 min, 4°). Protein concentrations were determined by the method of Lowry *et al.* (26).

Iodination of Virus and Fetal Calf Serum. Purified virus was disrupted with Nonidet P-40 (2% final concentration) and iodinated by a modification of the Hunter procedure (27). Usually 0.5 ml of virus (0.5-1.0 mg of protein) was mixed with 2 mCi of Na¹²⁵I (Amersham) and then 5 μl (250 μg) of chloramin T was added. The reaction was stopped after 2 min by the addition of 1 mg (in 5 μl) of sodium metabisulfite. Free iodine was removed on Sephadex G-25 columns. The specific radioactivity of the virus preparations was 5 to 10 × 10⁴ trichloroacetic-acid-precipitable cpm/μg of protein. In addition, fetal calf serum was iodinated and purified as above. Iodinated protein solutions were stored in aliquots at -20°.

Sera. Human sera were obtained from nine healthy colleagues and from 30 healthy donors at St. Bartholomew's Hospital. Several subjects donated blood at intervals to enable us to observe possible fluctuations in antibody titer. Umbilical cord human sera were obtained from normal deliveries. Hyperimmune goat sera specific for primate oncornavirus antigens were gifts of Dr. J. Gruber (Office of Logistics and Resources, NCI, Bethesda, Md.). These included different samples of anti-SSAV, anti-gibbon ape lymphoma virus (GALV), and anti-BEV sera and in some cases corresponding preimmunization sera. Additional sera were obtained from nonimmunized animals.

Rabbit hyperimmune sera specific for human blood group substances M and N, as well as human typing sera directed against other human blood group antigens, were purchased (Ortho Diagnostics, Raritan, N.J.). High titer antisera against immunoglobulin G (IgG) were produced by immunization of rabbits (with human or goat IgG) or goats (with rabbit IgG) (28). IgG fractions were prepared to electrophoretic purity by 35%

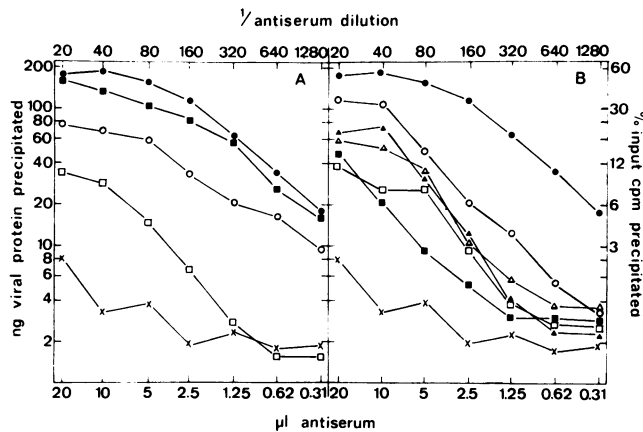


FIG. 1. Radioimmunoprecipitation of ^{125}I -labeled HL23V-1 virus proteins. (A) Titration of animal reference sera: (X) normal goat serum; (●) goat anti-SSAV; (○) goat anti-SSAV p30; (■) goat anti-GALV; (□) goat anti-BEV. (B) Titration of individual normal human sera: (○) serum 123; (▲) serum 124; (△) serum 125; (■) serum 126; (□) serum 127; (X) normal goat serum; (●) goat anti-SSAV. All data are taken from one experiment.

ammonium sulfate precipitation and DEAE-cellulose and Sephadex G-200 chromatography (29). Rabbit anti-chicken IgG was obtained from Cappel Laboratories (Downington, Pa.) or from Dr. H. Beug (Max-Planck-Institut, Tübingen). All sera were heat-inactivated (56° , 30 min) prior to use.

The titers are defined as ng of viral protein precipitated by 10 μl of serum and reported in comparison to those of hyperimmune animal sera. Therefore the radioimmunoprecipitation assays were always performed under conditions of antigen excess.

Radioimmunoprecipitation Assay. Many parameters influencing antigen-antibody binding were varied to find optimum conditions for the detection of antibodies of low avidity. These included: incubation time, buffers, concentration of carrier proteins and detergents, sedimentation of immune precipitates through fetal calf serum and/or 0.5–2.0% discontinuous sucrose gradients, and washing conditions. The version finally adopted is described here. (i) Carrier and washing buffer was isotonic phosphate-buffered saline or Tris-HCl buffers, pH 7.2, containing Ca^{++} and Mg^{++} and supplemented with 0.5 mg/ml of ovalbumin and 0.5% Nonidet P-40 (no EDTA was added). (ii) All solutions (antigens, antisera, buffer) were centrifuged ($12,000 \times g$, 30 min, 4°) just before assay to remove possible aggregates. (iii) Incubation was in siliconized tubes: 5 μl of virus antigen, 25 μl of carrier buffer, and 20 μl of test serum at 2-fold dilutions were incubated 3 hr at 37° before appropriate anti-IgG serum was added in 20-fold (vol/vol) excess over the test serum. Even with anti-IgG sera of high titer, a 10-fold (vol/vol) excess was required to precipitate >95% of the IgG in the test serum. This was determined in pilot experiments using ^{125}I -labeled IgG isolated from several human sera. (iv) Incubation was terminated after 18 hr at 4° by sedimentation of the immune complex ($200 \times g$, 10 min) followed by two washes in 0.5 ml of buffer at 4-hr intervals at 4° to allow for reaggregation of small, soluble immune complexes. (v) Radioactivity of sediments resuspended in sample buffer was assessed in a gamma scintillation counter.

Sodium Dodecyl Sulfate (NaDodSO₄)-Polyacrylamide Gel Electrophoresis. Sediments in sample buffer (0.08 M Tris-HCl, pH 6.8, 2% NaDodSO₄, 10% glycerol, 2 mM phenylmethylsulfonyl fluoride, 5% 2-mercaptoethanol, 0.2% bromphenol blue) were heated 4 min in a boiling-water bath and subjected

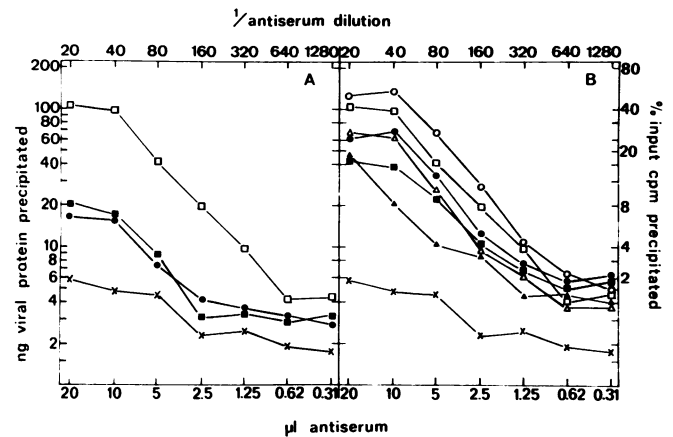


FIG. 2. Radioimmunoprecipitation of ^{125}I -labeled BEV virus proteins. (A) Titration of animal reference sera. (B) Titration of individual normal human sera. Symbols as in legend of Fig. 1. All data are taken from one experiment.

to NaDodSO₄-polyacrylamide gel electrophoresis (30). One-millimeter slab gels (12 cm length) with 7.5–12.5% acrylamide concentrations were run at 100 V, 30 mA. Gels were stained with Coomassie blue, dried, and put on x-ray films to locate radioactive virus proteins. After developing, films were scanned on a densitometer.

Competition Radioimmunoprecipitation Assays. A series of human sera were titrated for precipitating activity. The serum concentration precipitating 70% of the input viral antigen was used in competition assays. Sera were incubated (3 hr, 37°) with increasing dilutions of excess unlabeled viral antigen; then iodinated virus was added to compete for the antibodies followed by the standard radioimmunoprecipitation assay.

RESULTS

Sera from goats immunized with type-C primate oncornaviruses were used to determine optimal conditions for the radioimmunoprecipitation assay. At low serum dilutions, up to 60% of the input detergent-lysed HL23V-1 was precipitated by anti-SSAV and anti-GALV sera (Fig. 1A). Anti-SSAV p30 serum was also reactive, though to a lesser extent. Slight precipitation was also observed with anti-BEV serum. Radioimmunoprecipitation tests with human sera and HL23V-1 or SSAV yielded essentially identical results (Fig. 1B). Only 19 out of 39 sera from healthy donors showed easily detectable precipitating activity of >20 ng of virus protein using 10 μl (1:40 diluted) of serum. This contrasts with other reports where, using intact labeled virus, all tested human sera reacted with SSAV (ref. 16; discussed below).

The goat anti-BEV serum reacted to high titer against BEV proteins (Fig. 2A) as well as against proteins of RD114 (data not shown). Weak crossreactivity was again seen with anti-SSAV and anti-GALV sera. In contrast to results with HL23V-1 and SSAV type-C viruses, all human sera precipitated radioactivity from BEV (Fig. 2B) preparations. Titers were consistently higher against BEV than against HL23V-1 or SSAV.

There are several reasons why all of the input radioactivity was not precipitated: detergent treatment may destroy or modify antigenic determinants of virus proteins, the iodinated virus preparations usually contain some protein impurities, and the antibodies are not directed against all virus proteins (Fig. 3). The HL23V-1 or SSAV proteins precipitated by human serum were subjected to NaDodSO₄-polyacrylamide gel electrophoresis and autoradiography and were compared with the

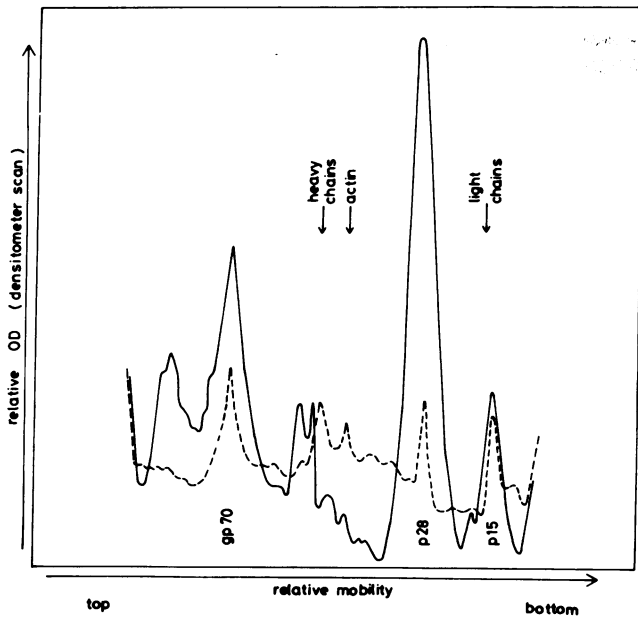


FIG. 3. Densitometer scan of iodinated SSAV viral proteins (—) and characteristic immune precipitates thereof with human serum 129 (---) after separation on 10% NaDodSO₄-polyacrylamide gels. A corresponding precipitation of the BEV polypeptides gp70, p28, and p15 is obtained with this serum. Purified p28 proteins of both SSAV and BEV can also be precipitated directly by many human sera (Thiel, Kurth, and Schäfer, unpublished data).

protein pattern of coelectrophoresed virus particles. Not all normal human sera precipitated the three bands comigrating with viral gp70, p28, and p15 proteins; some sera precipitated only gp70, others only p28 and p15.

Immunoglobulin G fractions precipitated equivalent amounts of SSAV and BEV as the corresponding volume of whole serum (Table 1). Avian sarcoma virus could not be precipitated. Additional evidence that the reaction is mediated by IgG was derived from the finding that heterologous anti-IgG serum was needed for immune lattice formation and successful precipitation of the otherwise soluble viral antigen-IgG immune complexes.

The average precipitating activities of animal and human sera with proteins from several viruses are summarized in Table 2. There was a weak reaction of the human sera with MuLV; in these cases, the only band seen after gel electrophoresis and

Table 1. Radioimmunoprecipitation of oncornavirus proteins by IgG fractions or whole sera

Serum*	Virus protein precipitated (ng)		
	ASV‡	SSAV	BEV
Serum 128†	4 (5)§	41 (46)	45 (38)
Serum 127†	9 (2)	53 (45)	63 (55)
Serum 126†	NT¶	22 (35)	39 (53)
Serum 123†	8 (6)	95 (88)	135 (122)
Pooled human IgG	NT	(39)	(71)
Normal goat serum	8 (9)	15 (9)	6 (8)
Goat anti-SSAV	11 (3)	188 (210)	16 (25)
Goat anti-BEV	7 (8)	37 (23)	97 (106)

* Serum (10 μ l) or corresponding amount of IgG (~60 μ g).
 † Individual healthy blood donors.
 ‡ Avian sarcoma virus.
 § Values in parentheses represent precipitation with IgG fractions isolated from the particular serum.

Table 2. Average radioimmunoprecipitation of oncornavirus proteins

Serum*	Virus protein precipitated (ng)†			
	MuLV	HL23V	SSAV	BEV
Goat sera				
Normal (3)	<10	<10	<10	<10
Anti-SSAV (2)‡	62 \pm 6	188 \pm 12	193 \pm 17	16 \pm 1
Anti-GALV (2)‡	NT§	134 \pm 14	142 \pm 14	18 \pm 1
Anti-BEV (2)‡	<10	37 \pm 2	21 \pm 4	97 \pm 8
Human sera				
Normal (41)	24 \pm 4	51 \pm 5¶	48 \pm 6¶	65 \pm 9
Cord (9)	NT	34 \pm 4	NT	NT

* The number of different sera samples tested is indicated within parentheses.
 † Nanograms of virus protein precipitated by 10 μ l of serum under conditions of antigen excess (200–600 ng) with standard deviation.
 ‡ Summary of >25 experiments.
 § Not tested.
 ¶ Because many normal sera do not show clear precipitation of HL23V-1 or SSAV proteins, the average value of 10 sera with detectable titers (>20 ng/10 μ l of serum) is given.

autoradiography was the major viral component p30. In addition, it was noted that the precipitating activity of human cord blood was entirely due to maternal IgG (Schmitt and Kurth, manuscript in preparation).

Competition radioimmunoprecipitation assays were performed with unlabeled SSAV and BEV to establish further that precipitation was due to an immunological reaction. As seen in Fig. 4A, unlabeled SSAV competed effectively with ¹²⁵I-labeled SSAV for the antibodies in goat anti-SSAV serum and in human sera. Unlabeled BEV was able to displace some ¹²⁵I-labeled SSAV at very high concentrations only. As depicted in Fig. 4B, unlabeled BEV competed effectively with ¹²⁵I-

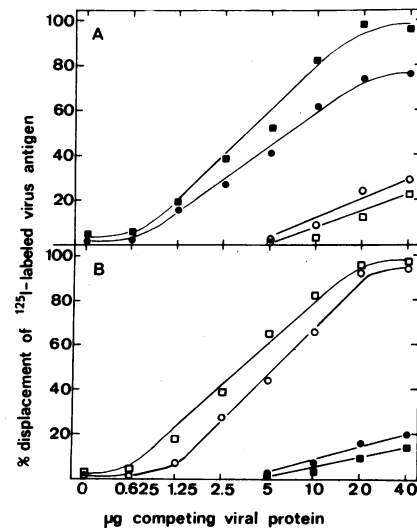


FIG. 4. Competition radioimmunoassays for SSAV and BEV proteins using detergent-disrupted SSAV or BEV as competing antigens. Assays contained 4 μ g (10⁵ cpm) of ¹²⁵I-labeled SSAV (A) or 4 μ g (4.9 \times 10⁴ cpm) of ¹²⁵I-labeled BEV (B) and the necessary amount of antisera to give 70% precipitation. Five human sera with average precipitating titers were pooled. (A) Competition for ¹²⁵I-labeled SSAV. Goat anti-SSAV reacting in the presence of unlabeled SSAV (■) or unlabeled BEV (□). Human test sera reacting in the presence of unlabeled SSAV (●) or unlabeled BEV (○). (B) Competition for ¹²⁵I-labeled BEV. Goat anti-BEV reacting in the presence of unlabeled BEV (□) or unlabeled SSAV (■). Human test sera reacting in the presence of unlabeled BEV (○) or unlabeled SSAV (●).

labeled BEV for anti-BEV, but unlabeled SSAV was not an effective competitor. These results confirm that the precipitations seen in the radioimmunoprecipitation tests represent immunological reactions specific to antigens closely related in both cases to the viruses used in the tests.

Cells in tissue culture may absorb or incorporate into their plasma membrane macroglobulins from serum in tissue culture medium (31). Oncornaviruses incorporate components of the host plasma membrane into their own envelopes. Therefore, natural human antibodies against serum proteins could be responsible for some part of the virus precipitating activity. This was excluded by the following findings: (a) 10 μ l of rabbit hyperimmune serum to fetal calf serum proteins precipitates only 10–20 ng of SSAV or BEV proteins; these values are sometimes obtained with normal rabbit sera also. (b) Goat anti-SSAV and anti-BEV sera show a low titer in immunodiffusion and radioimmunoprecipitation tests against calf serum proteins. In contrast, only two of nine sera from laboratory workers and no sera from random blood donors reacted with calf serum proteins in radioimmunoprecipitation tests, and titers were too low to be detected by immunodiffusion. (c) Concentrated calf serum proteins (1 mg) were unable to block the immunoprecipitation of SSAV and BEV by human sera.

Participation of natural human anti-Forsman antibodies in the radioimmunoprecipitation tests could be excluded because absorptions of human sera on packed sheep erythrocytes (1:5, vol/vol), which express Forsman antigen on their surface, did not lead to a significant reduction of antiviral titers. Of 11 sera absorbed with sheep erythrocytes, the average reduction in precipitating activity against SSAV or BEV viral proteins was 11%.

The possibility remained that certain viral antigens might crossreact with blood group antigens against which natural human antibodies are widespread. This appeared unlikely because there was no significant difference in average titers in radioimmunoprecipitation assays obtained with sera from donors having A, B, AB, or O blood groups. Furthermore, human typing sera specific for many human blood group antigens (A, A₁, B, K, k, S, s, P, Rh₀, hr', hr'', and Fy^a) did not precipitate viral antigens significantly more or less than control sera (range of human typing sera was 30–89 ng/10 μ l of serum against SSAV and 29–58 against BEV), nor did rabbit antihuman allotypes M or N sera precipitate more virus protein than normal rabbit serum.

DISCUSSION

Radioimmunoprecipitation assays revealed that most human sera react with proteins of C-type viruses of primate, and to a lesser extent, murine but not avian origin. The reactions appear to be mediated by immunoglobulin G because IgG purified from several human sera precipitated viral antigen as effectively as the corresponding volume of whole serum. Furthermore, heterologous anti-IgG serum was required for cross-linking and precipitation of the human IgG-virus antigen immune complexes. Snyder *et al.* (16) have also shown that human IgG and corresponding F(ab)₂-fragments can bind to SSAV particles. Human complement factors reported to lyse oncornaviruses (32) do not seem to participate in the reaction, since heat-inactivation of the sera did not alter the virus precipitating activity. Likewise, addition of fresh rabbit or guinea pig complement did not change the extent of precipitation (data not shown).

The natural human antibody titer against primate and murine type-C viruses is low. Therefore the radioimmunoprecipitation assay was modified to detect reproducibly the low

amount of antibodies that are also apparently of low avidity. All the human sera tested contained antibodies reacting to the immunologically related BEV and RD114 viruses. Not all sera clearly precipitated the HL23V-1 and SSAV viruses; this contrasts with recent findings of Snyder *et al.* (16), where all human sera showed precipitating activity to intact SSAV. It is remarkable that those human sera with highest antibody titers against BEV also possessed high anti-SSAV titers, even though the virus groups are immunologically distinct and the antigens did not compete for antibody to the other virus group in the human sera tested.

The precipitation of p28 and p15 proteins in the radioimmunoprecipitation assay of lysed virus is probably caused by specific anti-p28 and anti-p15 human antibodies. Forsman antigen, which can be induced in the host cell after oncornavirus transformation (34), was not involved in the radioimmunoprecipitation reactions, 12 human typing sera specific for defined human blood group antigens showed only average titers, and there was no significant participation of calf and fetal calf serum antigens. These data cannot exclude involvement of unknown heterophile antigens, but there is no evidence that internal virus structural proteins can associate with heterophile antigens.

NaDodSO₄-polyacrylamide gel electrophoresis analysis of precipitates of HL23V-1, SSAV, and BEV revealed the presence of gp70, p28, and p15 in the sediments. It is known from the murine oncornaviruses that these three proteins possess interspecies-specific determinants (33). We have no explanation why the gp70 of SSAV was not always found in the precipitates, as reported by Snyder *et al.* (16). The low precipitating titer against MuLV p30 may also be due to interspecies-specific determinants.

C-type viruses are transmitted either as endogenous proviruses integrated in the host genome, as is typical in mice, or as exogenous, infectious agents acquired horizontally and/or congenitally, as is typical of the oncogenic strains of virus in chickens, cats, and cattle. Molecular hybridization studies (35–37) indicate that proviral sequences homologous to SSAV or BEV are not represented to a significant extent in the DNA of normal human tissue. The serological studies presented here and by others provide indirect evidence that the infectious mode of transmission remains a real possibility in humans, and suggests that infection with an oncornavirus may be extremely widespread, though perhaps not typically pathogenic. Since human umbilical cord blood lacked detectable IgM antibodies reactive with viral antigens, we hope to gain further information by studying seroconversion in infants. It would be premature on the basis of results obtained thus far to draw any conclusions on the possible etiological role of C-type viruses in human disease.

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