and avian myeloblastosis virus (Fig. 3, arrow).

We have tested 37 samples of different human milks for a 60*S* to 70*S* RNA containing poly(A) sequences. Five, or 14 percent, were positive, ranging in particle counts from 1.0×10^6 to 4.0×10^7 particles per milliliter. A distinct peak at 35*S* was observed in a sixth sample. The procedures for particle purification were designed to eliminate free messenger RNA's and nuclei containing heterodisperse nuclear RNA, both of which contain poly(A). The fact that 86 percent of the milks tested were negative is an indication that the 14 percent positives are not due to normal RNA species of human lactating mammary tissue that are usually found in milk. Further, a diagnostic distinction can be made between the poly(A) stretches in the RNA of the human milk particle and those found in cellular messages. Gillespie *et al.* (*8a*) have shown by electrophoretic analysis that the poly(A) regions in cellular messages are extremely heterogeneous in size. In contrast, the poly(A) stretches in the 60*S* to 70*S* RNA of the human milk particle cluster sharply at a size range corresponding to 200 nucleotides (Fig. 3), a finding in agreement with that observed in the RNA tumor viruses (*5*). The RNA's of other mammalian viruses, such as poliovirus and eastern equine encephalitis virus, also contain poly(A) regions (*9*). However, neither the size of the RNA nor the length of the poly(A) region reported for these viruses corresponds to the results obtained here.

Numerous investigators (*10*), employing electron microscopic techniques, have reported the occasional presence of particles resembling RNA tumor viruses in human milk. These observations, however, depend not only on adequate numbers of particles, but particles that are morphologically intact. It has been shown (*11*) that human milks contain substances that destroy the morphological integrity of known RNA tumor viruses that are added. Viral particles, however, with outer membranes damaged or visually obscured by cellular debris, would still be detected by the poly(A) hybridization technique. The poly(A) assay depends only on the presence of an intact 70*S* RNA; thus, if only viral cores were present, a positive response would still be obtained (*12*). The poly(A) assay may also be used to detect defective mutants, such as RSV_α described by Hanafusa and Hanafusa (*13*), that do not contain reverse transcriptase. Finally, the poly(A) hybridization technique provides a useful method for quantitation of known and putative RNA tumor viruses.

J. SCHLOM, D. COLCHER
S. SPIEGELMAN
Institute of Cancer Research and
Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, New York 10032
S. GILLESPIE, D. GILLESPIE
Bionetics Research Laboratories, Bethesda, Maryland 20014

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Soluble Membrane Antigens of Lip and Cervical Carcinomas: Reactivity with Antibody for Herpesvirus Nonvirion Antigens

Abstract. *With the use of antibody for herpesvirus nonvirion antigens (not structural components of the virus) complement fixing reactivity has been shown for soluble membrane antigens separated from lip and cervical carcinomas but not for similar extracts from normal vaginal tissue or intestinal carcinoma. Neither the serum obtained from the guinea pig before hyperimmunization with the herpesvirus nonvirion antigen nor the antiserum of guinea pigs immunized with comparable uninfected cell extracts reacted with these tumor soluble membrane antigens. Since the above soluble membrane antigens could be specific markers for the presence of virus genome within the tumor cells, the findings could support an etiological role of herpesvirus in selected human malignancies.*

Antiserums to virus-specific, labile, nonvirion antigens in cells infected with herpes simplex virus (HSV) can be prepared by special absorption procedures (*1*). Specific, labile HSV-related soluble membrane antigens can be separated from invasive cervical tumor cells (*2*).

We now report that soluble cell membrane antigens extracted from carcinoma of the lip and from carcinoma of the cervix would react with antibody to HSV nonvirion antigens. An association of HSV subtype 2 with cervical cancer and of HSV subtype 1 with lip cancer has been suspected (*3*).

Normal vaginal tissue and invasive cervical, lip, and intestinal carcinomas were processed for preparation of soluble fractions from viable cell membranes (*2*). A 10 percent suspension of HEp_2 cells infected with the HSV type 2 (SAV strain) was used for the preparation of the virion and nonvirion antigens as well as for the antiserum to the partially purified HSV 2 (*2*). Virion and nonvirion antigens were extracted from HEp_2 cells and guinea pig kidney cells infected with the Schooler strain of HSV type 1; antiserums to these were prepared (*1*, *4*). The extracts of

cells harvested at 3 or 24 hours after infection with HSV and stored from 1 to 9 days at −80°C contained nonvirion and virion antigens. If these same extracts were stored at +4°C for 14 days, nonvirion antigens could not be demonstrated. The antiserum to the nonvirions after absorption with the harvested HSV infected cells, rendered free of nonvirion antigens as a result of storage at +4°C for 14 days, had antibody for labile nonvirion antigens but contained no antibody for stable virion antigens. The coded complement-fixation (CF) tests (*1*) were carried out in both laboratories. The method of absorption is described in reference (*1*).

In the soluble cell fractions 2 of the lip and cervix carcinomas were antigens that reacted with guinea pig antiserum to herpes nonvirion antigens, which had been absorbed with HSV type 1 virion antigen, but which did not react to rabbit antiserum for virion HSV_1 (Table 1). This reaction was highly specific since the guinea pig serum obtained from the animal before hyperimmunization with the HSV nonvirion antigen did not react with the soluble antigens from the tumor cell membranes. In addition, the guinea pig antiserums from animals immunized with comparable uninfected cell extracts did not react with the HSV nonvirion antigen or with the tumor soluble membrane antigen. Since the soluble membrane antigen fraction from the viable cells of the lip carcinoma was of low protein concentration, the corresponding cell membrane fraction from the cervical cancer was adjusted to a comparable protein concentration. The soluble antigen from the cervical cancer cell membranes reacted in the CF test with the serums of cervical cancer patients, and this reaction can be titrated precisely (*2*).

Soluble CF tumor antigen from another cervical carcinoma was measured with the same antiserum, absorbed in this experiment with HSV type 2 virion antigens; the positive reactivity could be titrated (see Table 2). When soluble antigens of a different tumor [intestinal cancer (*5*)] were tested against the same antiserum, they failed to react.

The soluble membrane fraction II from the cervical cancer cells and the nonvirion antigens obtained from HEp_2 and guinea pig kidney cells infected with HSV type 1 were separated by gradient polyacrylamide gel electrophoresis (*5*). One of the bands is in the same region and may be similar to the band seen in a sonicated herpesvirus type 2 preparation (Fig. 1).

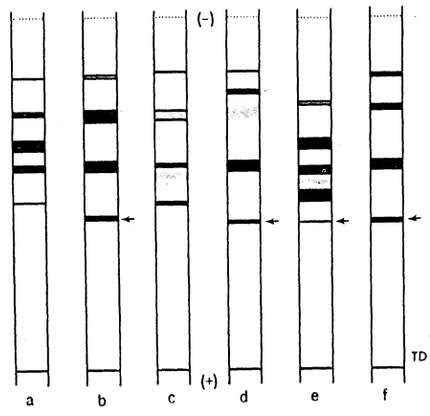

Fig. 1. Separation by gradient polyacrylamide gel electrophoresis. Four gel solutions were used in layers (10 percent, 7 percent, 4.75 percent, and 3.5 percent). Tracking dye (TD) was bromophenol blue. Materials separated were (a) sonicated HEp_2 cells; (b) HEp_2 cells containing virion and nonvirion HSV_1 antigens; (c) sonicated guinea pig kidney cells; (d) guinea pig kidney cells containing virion and nonvirion HSV_1 antigens; (e) sonicated herpesvirus type 2; (f) sonicated cervical cancer antigen. Arrows indicate bands in similar region for four of the preparations.

With the aid of specially prepared guinea pig antiserum from which the antibodies for HSV structural antigens had been absorbed, but which still reacted with HSV-induced nonvirion antigens (*1*), it was possible to show reactivity of this antiserum with tumor-specific, soluble membrane antigens separated from lip carcinoma and cervical cancer cells (*2*). The sharing of certain common sites, and about 50 percent DNA homology between HSV types 1 and 2 (*6*), lend credence to this study of cross-reacting CF antibody. Our data have special potential significance: If the tumor cells contain an antigen coded for herpesvirus, the specific antigens could be used directly for testing the serums of cancer patients. Antibodies to the separated cervical cancer antigens were found in unabsorbed serums from 95 percent of patients with cervical cancer and in 24 percent of unabsorbed serums from matched controls (*2*). Four unabsorbed cancer and six control serums (*2*) with no detectable antibody for herpesvirus were tested under code with HSV nonvirion antigen, normal vagina fraction II and cervical carcinoma fraction II. Four out of four serums of cancer patients reacted with HSV nonvirion antigen and with cancer antigen but not with control antigen, whereas control serums were CF negative with the three antigens. Results of further CF tests using HSV nonvirion antigen with unabsorbed

Table 1. Reactivity of antiserum to nonvirion HSV with soluble cell membrane antigens extracted from lip and cervical carcinomas.

Antigens	Treatment	Antigen reaction with antiserum to (reciprocals of CF units)	
		Nonvirion*	Virion†
HEp_2 cells, uninfected		0	0
HEp_2-HSV 1, 24-hour harvest‡	−80°C for 9 days	2	≥64
HEp_2-HSV 1, 24-hour harvest§	+4°C for 28 days	<1	64
Lip carcinoma (8 μg)‖	Soluble fraction 2	<2	<2
Cervix carcinoma (10 μg)‖	Soluble fraction 2	4	<2

* From heat-inactivated guinea pig serum used after absorption with HSV type 1 virion antigen. † From rabbit serum after infection with HSV and hyperimmunization. ‡ Contains virion and nonvirion HSV_1 antigen. § Contains only virion HSV_1 antigen. ‖ Amount of protein in the soluble fraction tested.

Table 2. Reactivity of antiserum to nonvirion HSV with HSV-infected HEp_2 cells and with soluble membrane antigens extracted from cancer cells.

Antigens	Treatment	Reaction with antiserum-nonvirion CF units*
HEp_2 cells-HSV 2, 24-hour harvest	Fresh †	4
HEp_2 cells-HSV 2, 24-hour harvest	2 weeks at +4°C ‡	<1
Normal vagina (16 μg)§	Soluble fraction 2	<2
Cervical cancer (15.5 μg)	Soluble fraction 2	4
(7.75 μg)		3
(3.87 μg)		1
(1.93 μg)		0
Intestinal cancer (16.5 μg)	Soluble fraction 2	<2

* From heat-inactivated guinea pig serum, absorbed with HSV type 2 virion antigen. † Contains virion and nonvirion HSV_2 antigens. ‡ Contains only virion HSV_2 antigens. § Protein concentration tested.

serums (*2*) containing antibody to HSV type 2 were confusing. If correctly absorbed human cancer serums should contain a specific tumor antibody reacting only with specific herpesvirus-coded nonvirion antigens, this finding would also help to prove that the tumor cell contains herpesvirus genetic information and indicate an etiological relationship between the HSV and the tumor.

ARIEL C. HOLLINSHEAD
Laboratory for Virus and Cancer Research, George Washington University Medical Center, Washington, D.C. 20037

GIULIO TARRO
Virologia Oncologica, Istituto di Clinica Medica I, Università di Napoli, Policlinico, Nàpoli 80138, Italy

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Familial Hypophosphatemic Rickets: Defective Transport of Inorganic Phosphate by Intestinal Mucosa

Abstract. *Uptake of inorganic phosphate is impared in intestinal mucosa from hemizygous males and heterozygous females with X-linked familial hypophosphatemic rickets. Considerable intrafamilial and interfamilial variation in uptake of inorganic phosphate is observed in affected patients. Uptake by normal mucosa is concentrative and energy-dependent, and is mediated by at least two systems with widely different affinities. These results lend direct support to the thesis that the primary metabolic disturbance in this disease results from impaired transport of inorganic phosphate in kidney and gut.*

A reduced serum concentration of inorganic phosphate (P_i) is the most constant biochemical hallmark of familial hypophosphatemic rickets (FHR), a human X-linked dominant trait. Affected hemizygous males exhibit hypophosphatemia and severe rickets; heterozygous females tend to have a higher serum P_i concentration and less severe bone disease (*1*). For more than 30 years investigators have sought a unifying biochemical and genetic defect which would explain the findings that characterize this disorder: hypophosphatemia, hyperphosphaturia, reduced intestinal absorption of calcium and phosphorus, and rickets (*2*).

Two conflicting theories of pathogenesis have evolved. One view holds that a primary defect in endogenous conversion of vitamin D to its active metabolite is responsible for intestinal malabsorption of calcium (*3*); this in turn leads to secondary hyperparathyroidism resulting in decreased renal tubular reabsorption of P_i, hypophosphatemia, and rickets. Recent evidence has weakened this argument. Hadded *et al.* reported that the concentration of 25-hydroxycholecalciferol in serum of patients with FHR was normal (*4*). Furthermore, Arnaud *et al.* (*5*) and Roof *et al.* (*6*) showed that serum parathyroid hormone concentrations were normal or reduced in affected patients, not increased.

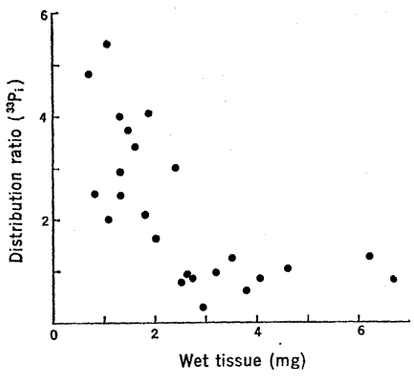

Fig. 1. Relation between weight of jejunal biopsy specimens and distribution ratio for radioactive inorganic phosphate ($^{33}P_i$). Specimens were obtained from eight controls and were incubated for 20 or 40 minutes at a $^{33}P_i$ concentration of 0.003 m*M*. Distribution ratios, corrected to 20 minutes of incubation, are expressed as counts per minute of $^{33}P_i$ per milliliter of cell water divided by counts per minute of $^{33}P_i$ per milliliter of incubation medium.

The alternate view proposes a primary membrane transport defect for P_i in the proximal renal tubule (*7*). This hypothesis has been challenged because it fails to explain either the capacity of calcium infusion to reverse the hyperphosphaturia (*8*), or the intestinal malabsorption of calcium. Glorieux and Scriver (*9*) have resolved the objection raised by calcium infusion studies. Their data indicate that renal tubular reabsorption of P_i is normally accomplished by two discrete mechanisms. The first is a parathyroid hormone–sensitive system responsible for reclamation of about two-thirds of filtered P_i; this system is defective in FHR. The second is a parathyroid hormone–resistant but calcium-responsive system that mediates reabsorption of the remaining P_i and accounts for the augmented P_i reabsorption observed in FHR patients during calcium infusion.

Intestinal calcium malabsorption in FHR can also be explained if one proposes that the functionally similar jejunal mucosal cell shares with the renal tubule cell the defect in phosphate transport (*10*). Such a primary disturbance in intestinal absorption of P_i might lead to the formation of insoluble calcium-phosphate complexes in the intestinal lumen, and, hence, to malabsorption of calcium. In vivo evidence for impaired intestinal absorption of P_i in FHR is derived from metabolic balance studies (*11*) and estimation of serum P_i during an oral phosphate tolerance test (*12*). Such in vivo studies, however, cannot distinguish primary intestinal malabsorption of P_i from malabsorption secondary to a defect in calcium transport. Therefore, the present in vitro studies of intestinal P_i transport were undertaken to examine P_i transport directly.

Inorganic phosphate flux and accumulation by rat intestine in vitro has been studied (*13*), but, to our knowledge, in vitro analysis of P_i transport