fluids of infected cells. Finally, Fig. 5D shows the negative outcome of the reaction with a purified human mycoplasma.

Table 1 contains a complete summary of the preparations tested for the 70*S* RNA–[^{3}H]DNA complex and their source. Of particular interest is the detection of the DNA–70*S* RNA complex in the plasma of the RIII mouse. Infectivity experiments have shown (*11*) that there are 10^5 to 10^6 more infectivity units per milliliter in RIII milk than in RIII plasma. Indeed the amount in plasma is so low that thus far immunological assays have failed to detect the presence of the MMTV antigen (*12*). However, the gradient assay of reverse transcriptase activity revealed the presence of the agent without difficulty.

None of the three human mycoplasmas tested showed any evidence of reverse transcriptase. This is of some technical interest since mycoplasma is a common contaminant of human milk.

There are two ways in which the initial assay can be performed. First, protein-free product is analyzed in a cesium sulfate gradient. Newly synthesized DNA is then looked for in the RNA region of the cesium gradient. This will occur whether or not the RNA being used as a template is partially broken either because of processing or because of the presence of ribonucleases in the material being tested. Second, product is analyzed in a velocity gradient. The presence of DNA in the 70*S* position of the gradient with subsequent analysis on cesium sulfate shows that an RNA molecule has been used as a template and that its size is 70*S*. By these devices one can demonstrate the presence of a 70*S* RNA molecule in a viral agent, which one cannot label isotopically or obtain in sufficient amounts to prepare RNA for examination by optical density in a velocity gradient.

The sensitivity and comparative certainty of the assay described for reverse transcriptase and the HMW-RNA make it a useful tool for exploring the presence of oncogenic RNA viruses in biological material of both animal and human origin.

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26 October 1971

Activation of Spontaneous Murine Leukemia Virus–Related Antigen by Lymphocytic Choriomeningitis Virus

Abstract. *Persistent infection with lymphocytic choriomeningitis (LCM) virus activates a phenotypic expression of murine leukemia virus–related antigen. NZB and (NZB × NZW)F_1 mice, which normally carry large amounts of Gross virus, and C57BL/6 and NZW mice, which normally carry little virus, were infected with LCM virus. All had Gross soluble antigen in their plasmas at 3 months of age, while noninfected matched controls of all strains did not. This effect was seen after infection with LCM virus that was tissue passed or plaque purified. Similarly, cultures of mouse-embryo fibroblasts produced Gross soluble antigen when infected with LCM virus, but noninfected cultures failed to do so.*

In an attempt to explain oncogenesis, it has been suggested that cancer is a natural event which is determined by spontaneous or induced derepression (or both) of an endogenous viral oncogene (*1*). Accordingly, all cells carry or contain information for the activation of oncorna viruses, with cancer expression being determined by both host genetic factors and events such as irradiation, carcinogenesis, and aging (*1, 2*). In addition to the above known modifying factors, we now report that chronic infection of mice or cultured mouse cells with lymphocytic choriomeningitis (LCM) virus activates a phenotypic expression of Gross antigen or antigens determined by the viral genome.

Inbred strains of NZW and NZB were originally obtained from the Laboratory Animal Center, Medical Council, Carshalton Surrey, England. Breeding was effected by brother-sister mating in our laboratory. The SWR/J and C57BL/6 inbred mice were obtained from Jackson Laboratories, Bar Harbor, Maine. The NZB and (NZB × NZW)F_1 hybrids have a relatively high leukemia incidence and carry large amounts of Gross virus, whereas C57BL/6, SWR/J, and NZW mice have low leukemia incidence; overt expression of Gross virus is generally absent in early life and only minimal in later life. Random testing of mice indicated that they were free of LCM, polyoma, and lactate dehydrogenase

Table 1. Appearance of Gross soluble antigen in the plasma of 3-month-old mice chronically infected with LCM virus. Mice were inoculated with either 1000 LD_{50} of LCM, 0.5 × 10^6 plaque-forming units (polyoma), or a 10 percent suspension of noninfected isologous brain tissue (control) within the first 15 hours of life.

Strain	Inoculum	Mice (No.)	GSA positive (%)
NZW	Control	29	3
	Polyoma	15	0
	LCM	25	84
NZB	Control	20	5
	Polyoma	10	0
	LCM	20	90
NZB × W	Control	20	5
	Polyoma	10	5
	LCM	10	90
C57BL/6	Control	25	0
	LCM	25	60

Table 2. Appearance of GSA in the plasma of 3-month-old NZW mice chronically infected with LCM virus.

Inoculum into newborn mice*	GSA positive†
NZW noninfected brain	1/20
NZW brain passed LCM	21/25
NZW brain passed LCM + antiserum to LCM	0/15
NZW brain passed LCM + antiserum to Gross antigen	9/11
LCM cloned virus Armstrong	7/10
LCM cloned virus $G_{35}A_1$	4/7

* Inoculation occurred within the first 15 hours of life. † Number of mice GSA positive per total number of mice studied.

virus infections. The LCM virus used was CA1371 Armstrong strain passed through mouse brain and obtained initially from Wallace Rowe (National Institute of Allergy and Infectious Diseases, Bethesda, Maryland); this virus was subsequently adapted to several murine strains in our laboratory. In addition, both $G_{46}A_1$ and Armstrong cloned, plaque purified LCM viruses were supplied by Charles Pfau (Department of Microbiology, University of Massachusetts). Polyoma virus was of a mouse kidney passage, small plaque forming type; it was provided by Karl Habel. The handling, titrating, and injecting of virus into newborn mice to produce chronic LCM and polyoma virus infections have been reported (*3*). Detection assay for Gross soluble antigen (GSA), with C57BL/6 antiserum to transplanted AKR spontaneous leukemia K_{36} cells and the method of conjugating rabbit antiserum to mouse IgG to fluoresceinated isothiocyanate have been described (*4*). Briefly, 0.5×10^6 unfixed EL_4 indicator cells were mixed with 50-μl samples of plasma or tissue culture supernatant to be tested for GSA. After being incubated for 30 minutes at room temperature and 30 minutes in the cold, the EL_4 cells were washed, mixed with antiserum to K_{36}, washed again, and then finally stained with rabbit antiserum to mouse IgG conjugated to fluorescein isothiocyanate. Both the antiserum to K_{36} and the fluoresceinated antiserum to mouse IgG had been previously absorbed with EL_4 cells to remove any nonspecific reaction.

The GSA detectable with C57BL/6 antiserum to K_{36} seems to be a type-specific Gross antigen sharing a common specificity with Gross cell surface antigen. This mouse typing serum for Gross antigen shows a specific reaction only with Gross cell surface antigen and not with antigens on the viral envelope (*5*). Hence, the viral genome may direct the synthesis of both the soluble antigen and the cell surface antigen, suggesting that soluble antigen may be cell surface antigen released from the cell surface into the tissue fluids.

The GSA was detected in the plasmas of most 3-month-old NZW, NZB, (NZB × NZW)F_1, and C57BL/6 mice chronically infected with LCM virus. Thus, the plasma from 84 percent of NZW, 90 percent of NZB and (NZB × NZW)F_1, and 60 percent of C57BL/6 3-month-old mice chronically infected with LCM virus were GSA positive as compared to only 3, 5, 5, and 0 percent of noninfected controls, respectively (Table 1). Activation of GSA formation occurred whether mice were infected with LCM virus at birth or transplacentally in utero. Chronic infection with polyoma virus was not associated with the appearance of GSA (Table 1).

Since all mice probably carry Gross virus in their tissues, it was important to be sure that the appearance of GSA was due to LCM virus infection. Three different kinds of experiments were made to exclude Gross viral contamination. First, LCM cloned, plaque purified viruses were inoculated into NZW mice at birth. At 3 months of age, 57 to 70 percent of these mice had GSA in their circulation, as compared to less than 3 percent of noninfected controls (Table 2). Second, LCM virus passed through brains of NZW mice was incubated with (W/Fu × BN)F_1 rat serum to W/Fu (C58NT)D that neutralizes Gross leukemia viruses (*6*), for 30 minutes at room temperature and inoculated into mice. The increased incidence of GSA persisted. However, a similar incubation with a guinea pig antiserum to LCM did alter the incidence (Table 2). Third electron microscopic examination of pooled LCM viruses that had undergone brain passage failed to reveal any oncorna virus particles.

Next, experiments in vitro were done to determine whether GSA was made by cultured mouse cells infected with LCM virus. Confluent monolayers of NZW, NZB, (NZB × NZW)F_1, and SWR/J embryo cultures were infected with LCM virus passed in isologous tissue culture (0.2 mouse intracerebral LD_{50} of infectious virus per cell). After incubation for 1 hour, the culture cells were washed with phosphate-buffered saline and then fed with Eagle's basic medium supplemented with glutamine and antibiotics and containing 5 percent fetal calf serum. Control cultures incubated with supernatants from isologous noninfected cultures were handled similarly. Results are presented in Table 3 and indicate that by day 14 only cultures infected with LCM virus produced significant amounts of GSA.

We have not yet determined whether other expressions of oncorna virus are present in mice or cultured cells infected

Table 3. Appearance of Gross soluble antigen with LCM infection in vitro. In the assay system EL_4 cells were first absorbed with the tissue culture supernatant and then incubated with C57BL/6 antiserum to K_{36} and rabbit antiserum to mouse IgG conjugated to fluorescein isothiocyanate, respectively. The prepartion was mounted on an acid-cleaned slide, covered, and sealed with paraffin; 200 cells were counted. When more than 20 percent of treated EL_4 cells showed GSA on their surfaces, tests were positive; Exp., experiment.

Strain	Inoculum	GSA detection (% positive cells)		
		Day 7	Day 14	Day 18
NZW*	Control	5	8	5
Exp. 1	LCM	8	38	44
Exp. 2	LCM	3	35	41
NZB*	Control	6	9	12
Exp. 1	LCM	5	41	56
Exp. 2	LCM	7	28	37
(NZB × NZW)F_1*	Control	6	6	13
Exp. 1	LCM	8	48	50
Exp. 2	LCM	13	35	27
SWR/J*	Control	2	3	5
Exp. 1	LCM	3	30	36
Exp. 2	LCM	6	24	31

* Embryo cell lines.

with LCM virus. In this regard, others have reported an association of lymphomatosis and tumor in mice chronically infected with LCM virus (*7*). In addition to the activation of Gross viral genome, LCM virus infection also potentiates the growth and yield of rabies virus (*8*) and, under certain circumstances, vesicular stomatitis virus (*9*). In contrast, LCM virus infectivity is not apparently enhanced by leukemia viral infections. Other experiments in our laboratory have shown that Gross, Moloney, or Rauscher viruses or cells infected by them do not increase LCM virus infectivity or yield of LCM virus produced.

Our results have several important implications. First, the phenotypic expression of the Gross viral genome may be activated by a chronic nononcogenic virus which can be vertically transmitted. This effect results in the production of GSA and occurs both in mice with a high as well as a low incidence of leukemia. Second, in addition to its oncogenic properties, oncorna virus participates in immunologically induced disease by virtue of its interaction with sensitized cells or antibodies (or both) produced by the host. In situations where these viruses are activated or enhanced, as by the LCM virus in our studies, their contribution to immunologic diseases might be expected to increase.

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10. Supported by PHS grants AI-09484, AI-07007, CA-10596 and a Violet June Kertell memorial grant for research on multiple sclerosis from the National Multiple Sclerosis Society. M.B.A.O. is recipient of career development award KO4 AI-42580 from the U.S. Public Health Service. Publication 519 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California.

11 June 1971

Central North Atlantic Plate Motions

Phillips and Luyendyk (*1*) have computed a pole of relative motion for the central North Atlantic for the past 40 million years on the basis of a detailed survey of the Atlantis fracture zone. The position of the pole was determined from the azimuth of the fracture zone at various points along the fracture zone by a technique similar to that of Morgan (*2*). The rate of opening was obtained by identification of anomalies in the vicinity of the fracture zone.

If the theory of plate motions is valid, it should be possible to use the pole obtained by Phillips and Luyendyk to describe other fracture zones at the North American–African plate boundary. Furthermore, if the pole and rate computed by Phillips and Luyendyk are used to rotate westward the positions of anomaly 13 on the east side of the ridge, the rotated anomalies should be along the lineation corresponding to anomaly 13 on the west side of the ridge. We find that their pole satisfies neither of these criteria. We have compared a small circle generated by their pole with the Kane fracture zone (*3*) and find that there is a serious discrepancy (Fig. 1) which indicates that the position of the pole is in error. We have also rotated anomaly 13 from the east side of the ridge (*4*) to the west using their pole and rate of opening and assuming an age of 38 million years for anomaly 13 (*5*). The rotated lineation is seen to diverge considerably from the western lineation 13.

This divergence also indicates that the position of the pole is in error. The fact that the lineations do not meet at any point means that the half-spreading rate of 1.3 cm/year computed by Phillips and Luyendyk for a latitude

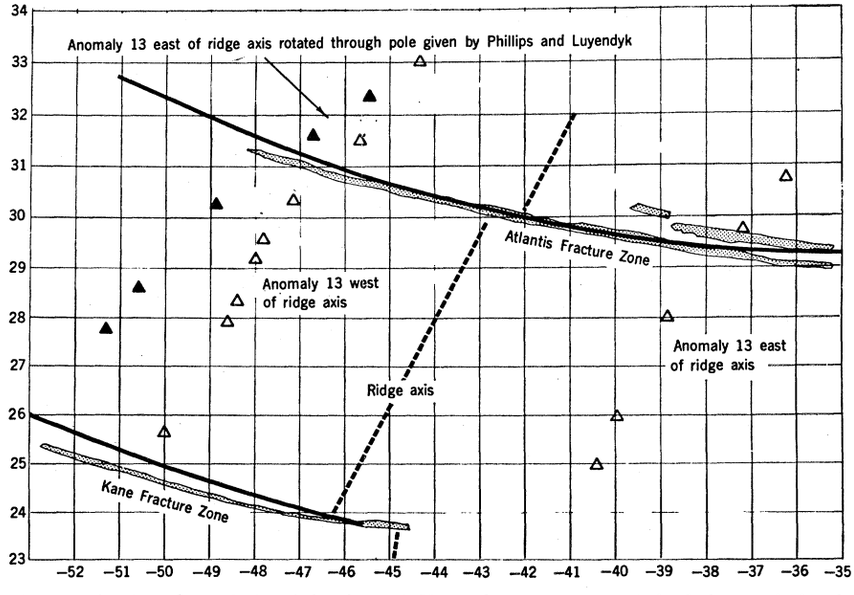

Fig. 1. The location of the Atlantis and Kane fracture zones (stippled areas) in the North Atlantic. The solid black lines are portions of small circles about the pole of rotation at 52.5°N, 34°W deduced by Phillips and Luyendyk. The open triangles give the present positions of anomaly 13. The solid triangles show the points from the east rotated to the west by use of the pole and rate of Phillips and Luyendyk (*1*).