PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 557–561 (1975)

Demonstration of Feline Leukemia Virus Antibody in Cats by Passive Hemagglutination (38583)

J. T. WANG AND K. M. LEE
(Introduced by D. W. Bruner)

Department of Microbiology, New York State Veterinary College, Cornell University, Ithaca, New York 14850

Sera of leukemic cats have been shown to contain little or no antibodies to feline leukemia virus (FeLV) as demonstrable by complement fixation (CF) or gel-diffusion (1–4). Using the passive hemagglutination (PHA) test, however, Sibal *et al.* (5) detected antibody in cat sera and concluded that the antibody was probably directed to a surface antigen of FeLV. This study was conducted using antigenic fractions of disrupted FeLV obtained by Sephadex gel filtration in PHA tests to elucidate the nature and significance of the hemagglutinating antibodies.

Materials and Methods. Virus preparations: The source of virus was chronically infected suspension cell cultures of F422 cell line (6, 7). The virus was precipitated from the culture fluid by the addition of 3% polyethylene glycol (w/v) (8), then purified by isopycnic centrifugation in sucrose gradients, and finally disrupted by Tween-ether or Triton-ether. Antigenic fractions were obtained on a Sephadex G-150 column by the method of Scolnick *et al.* (9): Briefly, virus suspensions were disrupted by stirring at 0° for 60 min in 1% (v/v) Triton X-100, and then extracted five times with ether (3:1) to remove the detergent; ether was then removed by nitrogen aeration. This material was filtered on a 2.5 × 90 cm Sephadex column eluted with 0.02 *M* Tris–HCl, pH 7.8 containing 0.30 *M* KCl. The flow rate was 10 ml/hr and 5 ml fractions were collected. Antigenic reactivity was monitored against rabbit antiserum by the conglutinating complement absorption (CCA) test (see below). The group-specific (gs) antigen was confirmed by immunodiffusion with specific rabbit antiserum (prepared against FeLV p30 by Dr. F. Noronha, Cornell University). The most antigenic three 5 ml fractions were pooled and used for coating tannic acid treated sheep red blood cells (SRBC) without concentration (5 ml/0.4 ml of packed SRBC). The focus-forming virus used for the neutralization test, MSV (FeLV), was originally given by Dr. P. J. Fischinger, NCI.

Immunization of animals with disrupted FeLV. Each of two rabbits was inoculated into the foot pad with 0.5 ml of emulsified virus consisting of one part Tween-ether disrupted virus and one part incomplete Freund's adjuvant. Every 2–3 wk the rabbits were given additional intramuscular injections of 1 ml emulsified virus. Three kittens were obtained from the Specific-Pathogen-Free colony of Cornell University, and each was hyperimmunized by subcutaneous injections of 0.5 ml emulsified virus followed by eight additional intramuscular 1 m injections at 1- to 2-wk intervals.

Sera. Sera from 14 cats with naturally occurring malignant lymphoma were provided by various Contractrs in the Virus Cancer Program from the National Cancer Institute.

Serologic tests. The procedure used in coating SRBC was that of Herbert (10). The dilution of antigen for the coating was determined by titration with serial dilutions of a rabbit specific immune serum against SRBC coated with varying amounts of virus. Each 0.1 ml of tanned packed called was exposed to 1.0, 0.75, 0.5, 0.25, 0.1, and 0.05 ml of virus, and the optimal dilution was defined as that volume of antigen which gave the highest antibody titer. Typically this was 0.25 ml of virus or 100–125 μg of viral protein or 1500–2000 COCAL units (11) per 0.1 ml packed SRBC. Tanned SRBC unexposed to virus and the goat antiserum that gave a PHA titer of 1:1024 with SRBC sensitized with disrupted FeLV were included in the test as controls.

The microtiter procedure of the CCA test, a modification of the methods described by Hole and Coombs (12, 13) and Coombs *et al.* (14), will be published in detail else-

where (Lee *et al.* manuscript in preparation). The CCA technique is similar to the CF test, except that horse complement is used instead of guinea pig complement and the indicator system is made up of SRBC and normal bovine serum as conglutinin. Appropriate doses of conglutinin, complement, and antigen (or antiserum) are determined in preliminary titrations. In a final test for measuring viral antigen, twofold dilutions of the antigen to be titrated are mixed with 2 units of complement and 4 units of antiserum. The mixture is incubated at 37° for 1 hr. Then the indicator system consisting of equal volumes of 0.5% SRBC suspension and 2 units of conglutinin is added. The unit volume of each ingredient is 25 μliters. After the final incubation at 37° for 30 min, the reactions are read without centrifuging the microtiter plate by examining the pattern of cells formed on the bottom of the well.

Gel-diffusion assays were made on microslides with 0.85% agar in barbital buffer at pH 8.6. The CF was carried out by the microtiter procedure of Sarma, *et al.* (11) with minor modifications. The focus reduction neutralization procedure was a modification of the method previously described (5). At least 100 MSV (FeLV) foci were counted in control plates, and a reduction of 90% or more was considered as positive.

Results. Rabbit antisera were absorbed with lyophilized cat cell homogenate and lyophilized fetal bovine serum until no reactivity to these antigens could be demonstrated by gel-diffusion. Similarly, cat antisera were exhaustively absorbed with lyophilized fetal bovine serum. The rabbit or cat antiserum, unabsorbed with feline or bovine antigens, gave a PHA titer identical to the absorbed serum, indicating that little, if any, antibodies to feline cells or fetal bovine serum were measured by the PHA reaction. The absorbed rabbit or cat antisera were then tested with Tween-ether disrupted FeLV by gel-diffusion, focus reduction neutralization, CF and CCA. The results of the tests and a comparison of the findings along with the results of PHA test are summarized in Table I. Following injections of virus, the rabbits developed antibody detectable by all methods. In contrast, cat sera gave a negative reaction when tested by gel-diffusion, only low and transient responses by CF, but one of the three produced neutralizing antibody. On the other hand, it was possible to detect antibodies from all cats by CCA and PHA tests. A general correlation was seen between CCA and PHA titers, although Cat 2 had the best CCA titer but the lowest PHA.

After the observation was made that antibodies could be detected in cat sera by PHA, the following experiments were conducted to determine whether such antibodies would have affinity for particular antigenic fractions of FeLV produced by Sephadex G-150 gel chromatography. As illustrated in Fig. 1, 2 OD_{280} peaks were obtained from the column and the gs antigen was eluted approximately at fraction numbers 50–67. The results of the PHA testing using these fractions are shown in Table II. It is apparent that rabbit sera were reactive against both fractions whereas cat sera reacted against the first peak eluants but not against the gs fraction.

Sera from 14 cats with naturally occurring leukemia were tested by focus reduction neutralization and by PHA using Tween-ether disrupted FeLV or the first peak eluants from the Sephadex G-150 column. As seen in Table III, for the 5 sera which had neutralizing titers only two had low PHA titers, and conversely, 9 PHA positive sera were negative for neutralizing antibody. This was contrary to the previous conclusion (5) that there was general agreement between PHA titers and neutralization results. It is significant to note that of 14 cats with leukemia one with a diagnosis of mast cell leukemia had a positive PHA titer at a dilution of 1:16 against the Sephadex second peak fraction of FeLV. Also interesting is that one hyperimmunized cat exhibited a low PHA titer (1:4) after the 13th week injection of disrupted FeLV.

Discussion. The results show that the rabbit can produce antibody to both Sephadex fractions of disrupted FeLV. The homologous host, the cat, however, responds poorly to the injections of disrupted FeLV and fails to mount an antibody response to probably a major virion antigen of FeLV.

TABLE I. IMMUNE RESPONSE TO FeLV OF RABBITS AND CATS MEASURED BY VARIOUS SEROLOGICAL TESTS

Schedule of FeLV injections (weeks)	Gel-diffusion		% focus reduction		Antibody titer: CF		CCA		PHA	
Rabbit	R-1	R-2	R-1	R-2	R-1	R-2	R-1	R-2	R-1	R-2
Preserum	–[a]	–	35	28	0[a]	0	4	4	0	0
2	–	+	37	86	16	64	32	32	64	16
4	+	+	100	98	32	64	32	64	128	32
6	+	+	100	98	NT[b]	NT	32	32	128	64
9	+	+	100	100	NT	NT	64	32	128	64
12	+	+	100	100	NT	NT	32	32	256	128

Cat	C-1	C-2	C-3	C-1	C-2	C-3	C-1	C-2	C-3	C-1	C-2	C-3	C-1	C-2	C-3
Preserum	–	–	–	<50	<50	68	0	0	0	4	8	4	0	0	0
1	–	–	–	<50	<50	<50	0	0	0	8	16	16	0	0	0
2	–	–	–	<50	<50	NT	0	0	2	32	16	32	4	0	16
3	–	–	–	<50	<50	NT	0	0	4	32	16	32	16	0	32
4	–	–	–	<50	<50	NT	0	0	2	32	32	32	16	0	16
5	–	–	–	<50	<50	<50	4	2	0	32	32	32	32	4	16
7	–	–	–	73	<50	NT	0	0	0	16	64	16	16	8	16
9	–	–	–	93	<50	NT	0	0	0	32	64	8	32	16	16
11	–	–	–	95	<50	<50	0	0	0	64	32	16	32	16	16
13	–	–	–	100	<50	<50	0	0	0	NT	128	NT	32	16	16

[a] –, 0: Negative reaction.
[b] NT: Not tested.

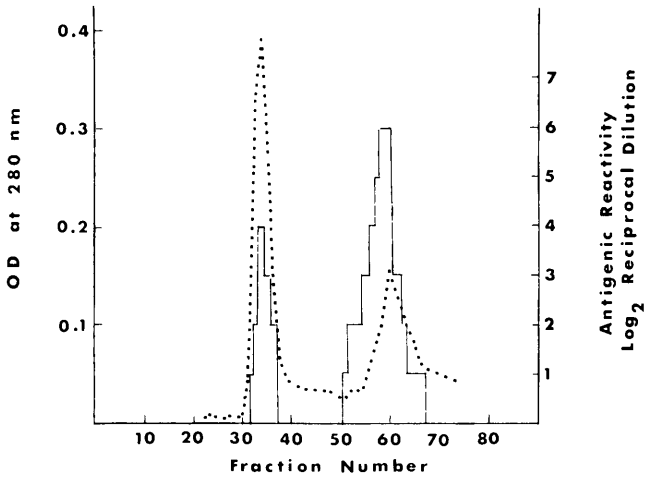

FIG. 1. Sephadex G-150 Gel Filtration of Triton-Ether Disrupted FeLV. Two OD_{280} peaks (---------) are shown. Antigenic reactivity (---------) of each 5 ml fraction was monitored against rabbit immune serum by the conglutinating complement absorption test. Highest dilutions fixing 2 units of horse complement in the presence of 4 units of the rabbit antiserum were measured.

The findings are consistent with the well accepted view that animals are refractory to developing antibody to the major virion antigen of homologous C-type particles (4). The data also suggest that CF or gel-diffusion tests are useless for the detection of FeLV antibodies in cat sera, probably because the tests are inherently insensitive in the cat system, or little or no antibodies, detectable by the tests, are produced in cats. Repeated injections of disrupted FeLV, however, elicited an antibody response in cats measurable by PHA or CCA. These tests should prove useful if the need for vaccinating cats and monitoring antibody response should arise.

The fact that all three hyperimmunized cats showed comparable PHA antibodies while only one developed demonstrable neu-

TABLE II. COMPARISON OF HEMAGGLUTINATING ANTIBODY RESPONSES OF RABBITS AND CATS TO ANTIGENIC FRACTIONS OF FeLV.

Schedule of FeLV injections (weeks)	Passive hemagglutination titer			
Rabbit	R-1		R-2	
	Peak I[a]	Peak II[a]	Peak I	Peak II
Preserum	0	0	0	0
2	2	0	0	0
4	4	0	4	8
6	4	0	4	8
9	16	16	4	16
12	32	32	32	16

Cat	C-1		C-2		C-3	
	Peak I	Peak II	Peak I	Peak II	Peak I	Peak II
Preserum	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	16	0	0	0	0	0
4	32	0	8	0	4	0
5	32	0	32	0	8	0
7	32	0	16	0	32	0
9	64	0	64	0	64	0
11	64	0	16	0	64	0
13	64	4	16	0	64	0

[a] Peak fractions eluted from a G-150 Sephadex column were used to sensitize sheep erythrocytes.

TABLE III. COMPARISON OF NEUTRALIZING AND HEMAGGLUTINATING ANTIBODIES TO FeLV IN SERA FROM LEUKEMIC CATS.

Registry No. of leukemic cats	Focus reduction neutralization at a serum dilution of		Passive hemagglutination titer with	
	1:10	1:2	TE[a]	Peak I[b]
120	+[c]		8	2
6040	+		0	0
7620	+		0	0
8650	+		2	4
9410	−	+	0	0
4000	−	−		32
5010	−		2	4
6760	−		4	2
6850	−	−	4	0
6930	−	−	8	2
6950	−	−	16	32
7040	−		8	0
7140	−		2	8
7150	−	−	2	0

[a] TE: Sheep erythrocytes coated with Tween-ether disrupted FeLV.

[b] Peak I: The first peak eluants from Sephadex G-150 column were used to sensitize sheep cells.

[c] At least 100 MSV(FeLV) foci were counted in control plates, and a reduction of 90% or more was considered as positive.

tralizing antibody led us to believe that PHA antibody may not be related to neutralizing antibody. Furthermore, we were not able to correlate PHA reactivity and neutralization by testing 14 sera from leukemic cats. It still remains to be determined, however, whether or not PHA antibodies in cat serum are directed against a surface antigen of the virus and are nonenutralizing.

It is of particular interest to note that one cat with the diagnosis of mast cell leukemia among 14 leukemic cats tested showed a gs antibody titer of 1:16 by PHA. Detection of gs antibody in spontaneously occurring leukemias has not been reported.

Summary. Two FeLV fractions from Sephadex G-150 gel filtration were used to sensitize RBC for the PHA test. When the cells were coated with the fraction from the second peak consisting mostly of a major gs antigen of FeLV, no antibodies could be detected either in hyperimmunized cat sera or sera from leukemic cats, whereas antibodies were readily detectable in immunized rabbits. Using cells coated with the first peak eluants or Tween-ether disrupted FeLV, PHA antibodies were detected in cat sera. There existed, however, one significant exception that a cat with the diagnosis of mast cell leukemia showed antibody against the second peak fraction. Little or no antibodies could be detected in cat sera by CF or gel-diffusion. There was some correlation between hemagglutinating antibodies and conglutinating complement absorbing antibodies, but these antibodies did seem to differ from neutralizing antibody.

This research was supported by NIH Grant No. CA-13085 and in part by Contract No. NIH-NCI-71-2508 within the Virus Cancer Program of the National Cancer Institute. We thank Dr. J. E. Post for supplying sera from leukemic cats, and Dr. C. L. Gries for his help in the performance of cat experiments.

1. Gardner, M. B., Rongey, R. W., Arnstein, P., Estes, J. D., Sarma, P., Huebner, R. J., and Rickard, C. G., Nature (London) **226,** 807 (1970).
2. Gardner, M. B., Rongey, R. W., Johnson, E. Y., DeJournett, R., and Huebner, R. J., J. Nat. Cancer Inst. **47,** 561 (1971).
3. Olsen, R. G., and Yohn, D. S., J. Nat. Cancer Inst. **49,** 395 (1972).
4. Huebner, R. J., Sarma, P. S., Kelloff, G. J., Gilden, R. V., Meier, H., Myers, D. D., and Peters, R. L., Ann. N.Y. Acad. Sci. **181,** 246 (1971).
5. Sibal, L. R., Fink, M. A., Plata, E. J., Kohler, B. E., Noronha, F., and Lee, K. M., J. Nat. Cancer Inst. **45,** 607 (1970).
6. Rickard, C. G., Post, J. E., Noronha, R., and Barr, L. M., J. Nat. Cancer Inst. **42,** 987 (1969).
7. De Noronha, F., Post, J. E., Norcross, N. L., and Rickard, C. G., Nature New Biol. **235,** 14 (1972).
8. Smith, R. E., and Bernstein, E. H., Appl. Microbiol. **25,** 346 (1973).
9. Scolnick, E. M., Parks, W. P., and Livingston, D. M., J. Immunol. **109,** 570 (1972).
10. Herbert, W. J., *in* "Handbook of Experimental Immunology," (Weir, D. M., ed.), 2nd ed., ch. 20. Blackwell Scientific Publications, Oxford (1973).
11. Sarma, P. S., Gilden, R. V., and Huebner, R. J., Virology **44,** 137 (1971).
12. Hole, N. H., and Coombs, R. R. A., J. Hyg. **45,** 490 (1947).
13. Hole, N. H., and Coombs, R. R. A., J. Hyg. **45,** 497 (1947).
14. Coombs, R. R. A., Coombs, A. M., and Ingram, D. G., "The Serology of Conglutination and its Relation to Disease," p. 168. C. C Thomas, Springfield, IL (1961).

Received March 5, 1974. P.S.E.B.M. 1975, Vol. 148.