nin-induced transformation of lymphocytes from POAG patients is inhibited by lower concentrations of prednisolone than in nonglaucomatous patients (*9*).

In an attempt to correlate corticosteroid responsiveness with the presence of HLA antigens, 25 POAG and 30 ocular normotensive patients were chosen at random from the population of the Glaucoma Center of Washington University and subjected to histocompatibility antigen typing and in vitro prednisolone inhibition of lymphocyte transformation (*2*, *9*). The POAG patients demonstrate the increased prevalences of B12 and B7 antigens previously described (Table 1) (*2*), and also the greater responsiveness of their lymphocytes to prednisolone (*9*) ($P < .001$). Whether from patients with POAG ($P < .02$) or from patients with ocular normotensive ($P < .02$), lymphocytes of individuals with HLA-B12 antigen respond to significantly lower concentrations of prednisolone than do those without this antigen. No apparent differences in prednisolone response are noted in either group of patients related to the presence of the HLA-B7 antigen. Relevant to these observations may be the recent finding that HLA-B12 differs from other A and B antigens in having valine instead of arginine in position 6 of the NH_2 terminal sequence (*10*).

The role of HLA-B12 antigens in the corticosteroid response of not only glaucomatous but also normal patients may be of importance. An analogous correlation of tissue antigens and glucocorticoid responsiveness is found in mice. The thymus cells of male mice with the $H2^a$ haplotype are reported to be more sensitive to the lytic action of corticosteroids in vivo and in vitro than are those mice with the $H2^b$ haplotype (*11*).

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References and Notes

1. S. R. Waltman, P. Palmberg, W. Newton, B. Becker, *Lancet* **1975-I**, 927 (1975).
2. D. H. Shin, B. Becker, S. R. Waltman, P. F. Palmberg, C. E. Bell, *Arch. Ophthalmol.*, in press.
3. D. H. Shin and B. Becker, *Am. J. Ophthalmol.*, in press.
4. D. H. Shin, M. A. Kass, B. Becker, *ibid.*, in press.
5. H. V. Bashir, J. M. MacQueen, D. B. Amos, J. J. Guinan, J. M. Johnston, B. Brotherton, B. Boettcher, G. J. Ashton, in *Histocompatibility Testing 1972*, J. Dausset and J. Colombani, Eds. (Munksgaard, Copenhagen, 1973), p. 311.
6. I. Mann and R. Roundtree, *Am. J. Ophthalmol.* **66**, 1020 (1968).
7. B. Becker, *Invest. Ophthalmol.* **4**, 198 (1965).
8. _____, S. M. Podos, C. Asseff, D. G. Cooper, *Am. J. Ophthalmol.* **75**, 73 (1973).
9. J. F. Bigger, P. F. Palmberg, B. Becker, *Invest. Ophthalmol.* **11**, 832 (1972).
10. J. Bridgen, D. Snary, M. F. Crumpton, C. Barnstable, P. Goodfellow, W. F. Bodmer, *Nature (London)* **261**, 200 (1976); C. Terhorst, P. Parham, D. L. Mann, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 910 (1976).
11. M. Pla, J. Zákány, J. Fachet, *Folia Biol.* **22**, 49 (1976).
12. This work was supported by grants EY 01167 and EY 00336 from the National Eye Institute, Bethesda, Md.

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Bovine Lymphosarcoma: Development of a Radioimmunologic Technique for Detection of the Etiologic Agent

Abstract. *A highly sensitive and specific radioimmunoassay has been developed for the major structural protein of an oncornavirus etiologically associated with bovine lymphosarcoma. This test can be used to identify cattle which have been exposed to the bovine leukemia virus and may thus develop or transmit the disease. Analysis of randomly obtained serums indicates that infection with this virus is widespread among cattle.*

A viral etiology to naturally occurring cancer has been established in several species including the chicken, mouse, cat, and gibbon ape (*1*). In these animals leukemia-causing type-C RNA viruses are transmitted as infectious agents. In recent years, evidence has accumulated that lymphosarcoma and leukemia of domestic cattle also have a viral etiology. Virus particles have been detected in lymphoid cells (*2*) and milk of leukemic cattle (*3*). Moreover, persistent lymphocytosis can be transmitted to newborn calves (*4*) and even sheep (*5*) by intravenous inoculation of cell-free filtrates prepared from tissue culture fluids of virus-producing cells. That the virus is horizontally transmitted under natural conditions has been suggested by the occurrence of a large number of lymphosarcoma cases in certain "high incidence" herds (*6*). Because this disease poses serious economic problems as well as an undefined hazard to humans, several countries have initiated disease eradication campaigns (*7*).

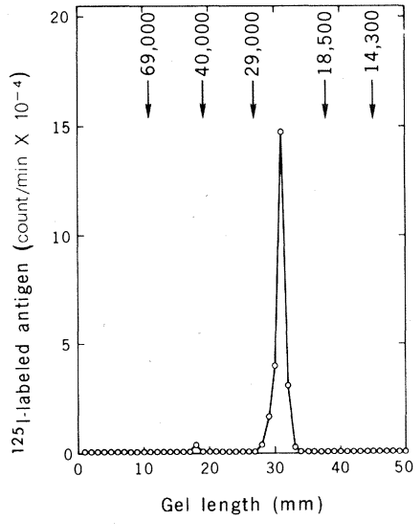

Fig. 1. Electrophoretic analysis of ^{125}I-labeled BLV p24. Samples of ^{125}I-labeled p24 [with a radioactivity of about 200,000 counts per minute (cpm)] were subjected to electrophoresis on 60 mm of 12 percent SDS-polyacrylamide gel at 1.0 ma per gel for 4 hours. After electrophoresis, the samples were either stained with Coomassie blue or sliced into 1-mm fractions and tested for radioactivity in a Searle gamma counter model 1285. Molecular weight standards used for calibration included bovine serum albumin (69,000); aldolase (40,000); carbonic anhydrase (29,000); β-lactoglobulin (18,500); and lysozyme (14,300).

A major problem in studying the epidemiology of bovine leukemia virus has been the lack of sensitive methods for detecting animals that have been exposed to the virus. Recently, immunofluorescence (*8*), immunodiffusion, and complement-fixation (*9*) tests for antibody to bovine leukemia virus (BLV) have been developed. However, clinical criteria are to a large extent still relied upon for the detection of infected animals, despite the obvious problem that such methods may detect only the small fraction of infected animals that develop overt disease (*10*, *11*). Radioimmunologic techniques have markedly increased the sensitivity with which viruses can be detected in a number of systems. In the present report we describe a radioimmunoassay for the detection and quantitation of serologic reactivity against the major structural protein of BLV. The results obtained with this method provide support for the etiologic association of BLV with lymphosarcoma of cattle and demonstrate that this radioimmunoassay can be used to detect animals that have been exposed to the virus.

The BLV was obtained from tissue culture fluids of chronically infected fetal lamb spleen cells (*12*), and was concentrated 1000-fold by centrifugation in a su-

crose density gradient. The major structural protein of BLV, p24 (molecular weight 24,000), was isolated by phosphocellulose column chromatography. About 5 mg of virus was disrupted by sonication in 0.05*M* tris-HCl (*p*H 9.0) buffer containing 1 percent Triton X-100. After 15 minutes of incubation at room temperature, samples were clarified by centrifugation at 100,000*g* for 30 minutes, dialyzed for 18 hours against 0.01*M* *N*,*N*-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid, *p*H 6.5, 1.0 m*M* EDTA, 0.1 percent Triton (BET buffer) and applied to a 1.6 by 5.0 cm Whatman P11 phosphocellulose column (Reeve Angel) equilibrated with BET buffer. The column was washed with 50 ml of BET buffer, and the bound proteins eluted with a 100-ml linear gradient of 0.0 to 1.0*M* KCl in BET buffer at 4°C. Fractions containing a single protein band with a molecular weight of 24,000, as determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (*13*), were pooled, and 0.05-ml portions were labeled with ^{125}I by the chloramine T method (*14*). The ^{125}I-labeled BLV p24 migrated as a single radioactive peak with a mass of about 24,000 daltons (Fig. 1).

As shown in Fig. 2, goat antiserum prepared against detergent-disrupted BLV precipitated the ^{125}I-labeled BLV p24 probe to the 50 percent level at a titer of over 1 : 10,000, and to a final extent of greater than 90 percent at a higher serum concentration. That BLV-infected but not uninfected bovine or fetal lamb cells competed in this reaction showed that the ^{125}I-labeled protein was specific for BLV. Such specificity was further indicated by the lack of detectable binding of ^{125}I-labeled BLV p24 by high-titered antiserums to previously described RNA tumor viruses (Fig. 2). These findings provide evidence for the specificity of binding of BLV p24 by antiserum to BLV and confirm and extend previous studies indicating that the major protein of BLV lacks cross-reactivity with other known mammalian RNA tumor viruses (*15*).

The immunoassay for BLV p24 was applied to the detection of serologic reactivity against BLV in defined herds so that the efficiency of this procedure for the detection of animals exposed naturally to BLV could be determined. Serums assayed without knowledge of their identities were obtained from cattle clinically diagnosed as having lymphosarcoma and from two herds (isolated from each other) at the Animal Disease Research Institute (ADRI), Ottawa, Canada. One herd has a high incidence of lymphosarcoma (high leukosis herd) (*11*); the other, designated a minimal disease herd, has shown no evidence of persistent lymphocytosis or overt disease during this same period (*11*).

As summarized in Table 1, serums obtained from 25 (out of 25) cases of adult bovine lymphosarcoma were serologically reactive against BLV p24. About 50 percent of these serums showed titers greater than 1 : 10,000, and in several instances titers as high as 1 : 50,000 were measured. In the high leukosis herd, serums from 17 (out of 20) cattle, including three animals showing evidence of clinical disease, demonstrated serologic reactivity against BLV by radioimmunoassay. Antiserum titers varied from 1 : 40 to 1 : 16,000. These findings indicate that a high proportion (> 95 percent) of serums from leukemic cattle contained antibody to BLV p24 as determined by the radioimmunoassay. In contrast, none of the serums obtained from the minimal disease herd showed detectable reactivity against BLV. As a control for the specificity of the test, BLV-reactive serums were shown to lack precipitating activity against the ^{125}I-labeled major antigens of representative type C virus isolates of the mouse and woolly monkey (*16*) (data not shown).

Serums from nearly 400 clinically normal cattle were next examined for exposure to BLV. These included samples from randomly selected dairy cattle from several different herds in Ontario, Canada, and Maryland. The results (Table 1) demonstrate that 10 percent of the animals tested exhibited antibody to BLV. Titers as high as 1 : 12,000 were observed. These findings suggest that the frequency of infection with BLV in cattle may be much higher than is clinically rec-

Table 1. Detection of antibody to ^{125}I-labeled BLV p24 in cattle from herds with different incidences of lymphosarcoma. Immunoprecipitation of ^{125}I-labeled BLV p24 was measured as described in the legend to Fig. 2. Serum titers are expressed as the reciprocal of the highest dilution capable of binding 20 percent of the ^{125}I-labeled BLV p24 and represent mean values of three separate determinations. The maximum binding represents the percentage of binding at the lowest serum dilution tested (1 : 10). Background precipitation of BLV p24 in the absence of antibody was never greater than 5 percent.

Animal status or source	Number of animals			Range of	
	Tested	Clinically affected	With antibodies to ^{125}I-labeled BLV p24	Antiserum titers	Maximum binding
Lymphosarcoma cases	25	25	25	100 to 50,000	82 to 99
Leukosis herd	20	3	17	40 to 16,000	70 to 92
Minimal disease herd	15	0	0		
Randomly selected normal animals					
Ontario, Canada	200	0	9	20 to 10,000	74 to 90
Maryland	180	0	16	20 to 12,000	71 to 92

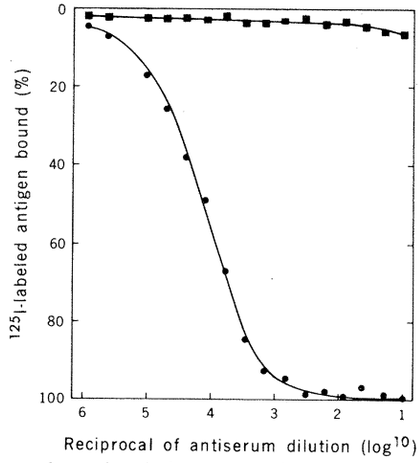

Fig. 2. Comparison of the abilities of antiserums to known RNA tumor viruses to bind ^{125}I-labeled BLV p24. Serums were incubated for 3 hours at 37°C and a further 18 hours at 4°C in 0.2-ml reaction mixtures containing ^{125}I-labeled BLV p24 (approximately 10,000 cpm), 0.01*M* tris-HCl, *p*H 7.8, 0.01*M* EDTA, 0.4 percent Triton X-100, 1 percent bovine serum albumin, and 0.01*M* NaCl. After the addition of 0.025 ml of antiserum to immunoglobulin G to each tube to precipitate the antigen-antibody complexes, reaction mixtures were incubated a further 1 hour at 37°C and 3 hours at 4°C. One milliliter of cold 10 m*M* tris-HCl, *p*H 7.8, and 10 m*M* NaCl buffer containing 0.1 percent Triton X-100 was added to each tube, samples were centrifuged for 15 minutes at 2500 rev/min, supernatants were aspirated, and the radioactivity in the precipitates determined. The results for goat antiserum to BLV are shown by circles; goat antiserums to woolly monkey type C virus, Rickard strain of feline leukemia virus, Rauscher murine leukemia virus, *P. cynocephalus* baboon virus, and Mason-Pfizer monkey virus, as well as rabbit antiserum to mouse mammary tumor virus (*20*) and a normal bovine serum (*21*) were also tested and the results are indicated by squares.

ognized. Analysis of human serums from 150 individuals in contact with cattle either as a result of working in slaughterhouses, or on dairy farms, has revealed no detectable antibody to BLV p24.

The incidence of bovine lymphosarcoma is currently estimated as around 20 per 100,000 for dairy cattle, and somewhat lower for beef cattle (*6*). Several countries have undertaken leukosis eradication campaigns or have attempted to eliminate the importation of cattle and semen from any but leukosis-free herds (*7*). This is primarily because of evidence that BLV can be spread horizontally (*6, 17*) and because epidemiologic studies indicate that in certain situations the incidence of bovine lymphosarcoma may be increasing (*18*). While the potential hazard of this virus to humans has not been fully evaluated, one recent report has indicated the development of leukemia in chimpanzees fed milk from clinically diseased cattle (*19*).

Serologic techniques, including immunodiffusion, complement fixation (*9*), and fluorescent antibody techniques (*8*) for the detection of BLV infection have offered important adjuncts to current clinical methods of disease detection which rely primarily upon hematological tests for persistent lymphocytosis. Comparison of existing immunologic methods with those described here are currently in progress. However, radioimmunoassays have in the past invariably proved to be more sensitive and specific for antibody detection than other immunologic procedures. The radioimmunoassay for BLV described herein should be useful in future epidemiologic studies in which the magnitude of BLV infection in cattle must be ascertained. Moreover, it should now be possible to estimate more thoroughly the potential hazard of this virus to humans through radioimmunologic analysis of serums from humans most likely to be at risk.

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References and Notes

1. L. Gross, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2013 (1974); M. M. Lieber and G. J. Todaro, in *Cancer: A Comprehensive Treatise*, F. F. Becker, Ed. (Plenum Press, New York and London, 1975), vol. 2; S. A. Aaronson and J. R. Stephenson, *Biochim. Biophys. Acta. Rev. Cancer*, in press.
2. J. M. Miller, L. D. Miller, C. Olson, K. G. Gillette, *J. Natl. Cancer Inst.* **43**, 1297 (1969); S. K. Dutta, V. L. Larson, D. K. Sorensen, V. Perman, A. F. Weber, R. F. Hammer, R. E. Shope, Jr., in *Comparative Leukemia Research*, R. M. Dutcher, Ed. (Karger, New York, 1970), p. 548; T. G. Kawakami, A. L. Moore, G. H. Theilen, R. J. Munn, in *ibid.*, p. 471.
3. R. M. Dutcher, E. P. Larkin, R. R. Marshak, *J. Natl. Cancer Inst.* **33**, 1055 (1964).
4. L. D. Miller, J. M. Miller, C. Olson, *ibid.* **48**, 423 (1972).
5. C. Olson, L. D. Miller, J. M. Miller, H. E. Hoss, *ibid.* **49**, 1463 (1972); M. J. Van Der Maaten and J. M. Miller, *Bibl. Haematol. (Pavia)* **43**, 377 (1976).
6. J. E. Croshaw, Jr., D. A. Abt, R. R. Marshak, W. C. D. Hare, J. Switzer, J. Ispen, R. M. Dutcher, *Ann. N.Y. Acad. Sci.* **108**, 1193 (1963); G. H. Theilen, R. D. Appleman, H. G. Wixom, *ibid.*, p. 1203; G. Migaki, *Natl. Cancer Inst. Monogr.* **32**, 121 (1969).
7. H. J. Bendixen, *Off. Int. Epizoot. Bull.* **62**, 675 (1964).
8. J. F. Ferrer, L. Avila, N. D. Stock, *Cancer Res.* **32**, 1864 (1972).
9. J. M. Miller and C. Olson, *J. Natl. Cancer Inst.* **49**, 1459 (1972); J. M. Miller and M. J. Van Der Maaten, *ibid.* **53**, 1699 (1974).
10. H. J. Bendixen, *Ann. N.Y. Acad. Sci.* **108**, 1241 (1963).
11. H. Tabel, S. Chander, M. J. Van Der Maaten, J. M. Miller, *Can. J. Comp. Med.* **40**, 350 (1976).
12. M. J. Van Der Maaten, J. M. Miller, A. D. Boothe, *J. Natl. Cancer Inst.* **52**, 491 (1974).
13. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
14. F. C. Greenwood, W. M. Hunter, J. S. Glover, *Biochem. J.* **89**, 114 (1963).
15. H. C. McDonald, D. C. Graves, J. F. Ferrer, *Cancer Res.* **36**, 1251 (1976); R. V. Gilden, C. W. Long, M. Hanson, R. Toni, H. P. Charman, S. Oroszlan, J. M. Miller, M. J. Van Der Maaten, *J. Gen. Virol.* **29**, 305 (1975).
16. J. R. Stephenson and S. A. Aaronson, *J. Virol.* **12**, 564 (1973).
17. C. E. Piper, D. A. Abt, J. F. Ferrer, R. R. Marshak, *Cancer Res.* **35**, 2714 (1975).
18. S. M. Stamatovic and M. J. Jovanovic, *Bibl. Haematol. (Pavia)* **31**, 194 (1968).
19. H. M. McClure, M. E. Keeling, R. P. Custer, R. R. Marshak, D. A. Abt, J. F. Ferrer, *Cancer Res.* **34**, 2745 (1974).
20. Obtained from R. Wilsnack through the Office of Resources and Logistics, National Cancer Institute.
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22. Supported in part by contracts N01-CP-61024 and N01-CP-4-3254 of the Virus Cancer Program of the National Cancer Institute. We thank G. Caldwell, Communicable Disease Center, Atlanta, Georgia, for supplying human serums. S. Devare is a visiting fellow of the National Institutes of Health. We thank M. Van Der Maaten and J. Miller of USDA, Ames, Iowa, for providing the BLV-infected fetal lamb spleen cultures.

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Field Focusing Nuclear Magnetic Resonance (FONAR): Visualization of a Tumor in a Live Animal

Abstract. *A nuclear magnetic resonance (NMR) image of a tumor in a live animal is reported. The field focusing NMR method or* FONAR *process that now achieves the tumor outline is described.*

Since the introduction of the nuclear resonance technique for detecting cancer by Damadian (*1*), many other investigators (*2, 3*) have extended the observation, including Weisman *et al.* who demonstrated its utility in vivo by detecting a tumor on the tail of a live mouse (*3*). The method of Damadian, originally conceived for the detection of internal neoplasms in humans, achieved its objective by focusing the nuclear magnetic resonance (NMR) signal within the interior of the live host. The focused NMR signal was thereby externally directed to any internal location in the live subject for data acquisition. The focusing NMR technique (called FONAR) was developed in 1972 (*4*).

FONAR should be distinguished from various nonfocusing methods that have appeared since. A promising modification of the projection analysis methods is the Fourier transform imaging technique by Kumar, Welti, and Ernst (*5*). Focusing NMR permits the operator to externally direct the NMR spot to the anatomic site of interest for firsthand inspection of the signal characteristics at a suspicious locus. Furthermore, field focusing allows the NMR signal behavior of each anatomic region to be continuously monitored during the data acquisition phase of the FONAR imaging process.

In principle, focusing NMR is achieved by field regulation of the spatial boundaries of a signal-producing region inside a sample. This is accomplished by shaping the magnetic fields (H_0 and H_1) across the entire sample so as to construct a small resonant window within the sample, such that the ratio of the spin moment of the nucleus to its gyric moment is everywhere satisfied by the static and time varying H fields and is everywhere dissatisfied beyond its boundaries. In practice, both the static H_0 field and the inductive component of the radio frequency (rf) field (H_1) are shaped. The

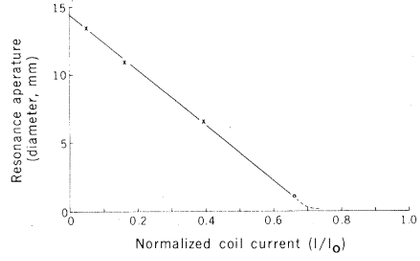

Fig. 1. Resonance aperture versus coil current. The o point shown is the extrapolated normalized current value for a 1-mm aperture.