In contrast, the partially purified peptide extract obtained from the intestine of the elasmobranch *Scyliorhinus*, which we refer to as fraction 13, contains a component or components that show potent stimulatory activity in the rectal glands of at least three widely differing species of elasmobranch. This component probably has a major role in the control of rectal gland secretion in vivo and is clearly responsible for determining the secretion rate. Preliminary experiments suggest that this component is restricted to the intestine, as similar extracts prepared from the stomach of *Scyliorhinus* were not active in the assays described. Although the constituents of fraction 13 are unknown, preliminary evidence based on methanol solubility and retention times on octadecyl silane reversed-phase HPLC with acetonitrile-trifluoroacetic acid gradients indicates that the peptide (or peptides) has properties distinct from those characteristic of VIP. In addition, radioimmunoassay of fraction 13 showed negligible quantities (<1 fmol/μg) of VIP-like immunoreactivity. Although its precise identity must await further purification and sequencing, we propose the name "rectin" for this putative peptide hormone.

TREVOR J. SHUTTLEWORTH
Department of Biological Sciences, University of Exeter, Exeter EX4 4PS, United Kingdom, and *Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672*
MICHAEL C. THORNDYKE
Department of Zoology, Bedford College, University of London, Regents Park, London NW1 4NS, United Kingdom

References and Notes

1. J. W. Burger and W. N. Hess, *Science* **131**, 670 (1960); J. W. Burger, *Physiol. Zool.* **35**, 205 (1962); *ibid.* **38**, 191 (1965).
2. J. S. Stoff, P. Silva, M. Field, J. Forrest, A. Stevens, F. H. Epstein, *J. Exp. Zool.* **199**, 443 (1977); P. Silva, J. S. Stoff, M. Field, L. Fine, J. N. Forrest, F. H. Epstein, *Am. J. Physiol.* **233**, F298 (1977).
3. T. J. Shuttleworth and J. L. Thompson, *J. Exp. Zool.* **206**, 297 (1978); *J. Comp. Physiol.* **136**, 39 (1980).
4. ———, *J. Comp. Physiol.* **140**, 209 (1980).
5. T. J. Shuttleworth, *J. Exp. Zool.* **221**, 255 (1982).
6. J. S. Stoff, R. Rosa, R. Hallac, P. Silva, F. H. Epstein, *Am. J. Physiol.* **237**, F138 (1979).
7. T. J. Shuttleworth, *J. Exp. Biol.* **103**, 193 (1983).
8. V. Mutt, in *Gut Hormones*, S. R. Bloom, Ed. (Churchill-Livingstone, Edinburgh, 1978), p. 21.
9. T. J. Shuttleworth, unpublished observations.
10. J. Hannafin, D. Friedman, E. Kinne-Saffran, R. Kinne, *Bull. Mt. Des. Is. Biol. Lab.* **22**, 69 (1982).
11. R. Solomon, M. Taylor, J. Stoff, P. Silva, F. H. Epstein, *ibid.* **21**, 16 (1981).
12. We thank R. Dimaline, Liverpool University Physiological Laboratory, for the radioimmunoassay, and P. Bevis and J. L. Thompson for technical assistance. Supported by Science and Engineering Research Council grants GR/B/67063 and GR/C/41777 (to T.J.S.), GR/B/68787 (to M.C.T.), and the Royal Society Scientific Investigations Fund (to M.C.T.).

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Antibodies to the Core Protein of Lymphadenopathy-Associated Virus (LAV) in Patients with AIDS

Abstract. *Lymphadenopathy-associated virus (LAV), a human T-lymphotrophic retrovirus isolated from a homosexual man with lymphadenopathy, has been causally associated with acquired immunodeficiency syndrome (AIDS). A sensitive and specific radioimmunoprecipitation test was developed for the detection of antibodies to the major core protein of LAV, p25 (molecular weight 25,000). Antibody to LAV p25 was found in the serum of 51 of 125 AIDS patients, 81 of 113 patients with lymphadenopathy syndrome, 0 of 70 workers at the Centers for Disease Control (some of whom had handled specimens from AIDS patients), and 0 of 189 random blood donors. Of a group of 100 homosexual men from San Francisco whose serum was obtained in 1978, only one had antibody to LAV p25; in contrast, of a group of 50 homosexual men in the same community whose serum was obtained in 1984, 12 had antibodies to LAV p25.*

One of the first indications that a human retrovirus might have a role in the etiology of AIDS was the finding of an increased prevalence, in patients with AIDS and lymphadenopathy syndrome (LAS), of antibodies to membrane antigens of human T-cell leukemia virus type I (HTLV-I) infected lymphocytes (*1*). HTLV-I had been frequently isolated from patients with mature T-cell malignancies and is etiologically associated with adult T-cell malignancy endemic in southern Japan, the Caribbean islands, and Africa (*2*, *3*). However, the rarity of HTLV-I isolates from AIDS and LAS patients and the low titer of antibodies to HTLV membrane antigens, together with the low prevalence of antibodies to virion core protein antigens of HTLV-I (*4*), suggested that cross-reacting antibodies to a related retrovirus was responsible for the initial observations. At the same time, a T-lymphotrophic retrovirus, subsequently termed lymphadenopathy-associated virus (LAV), was described (*5*). This cytopathic retrovirus was isolated from a homosexual man with lymphadenopathy. That this virus is linked to AIDS is suggested by (i) the presence of antibody to the virus in AIDS and LAS patients (*6*), (ii) the repeated isolation of the virus from AIDS and LAS patients (*6*), and (iii) the isolation of the virus from a blood donor–recipient pair of AIDS patients (*7*). Another T-lymphotrophic retrovirus, termed HTLV-III, has been isolated repeatedly in similar circumstances and antibodies to this virus have also been found in a high proportion of AIDS and LAS patients (*8*). Although comparative studies of LAV and HTLV-III have not been reported, it is possible that the two viruses are the same.

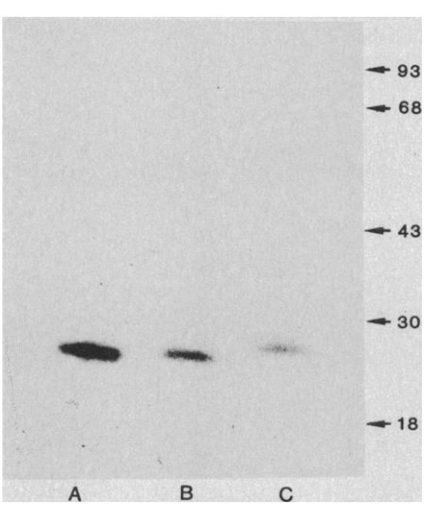

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of ^{125}I-labeled core proteins of (lane A) HTLV-I, (lane B) HTLV-II, and (lane C) LAV. HTLV-I p24 was purified from HTLV-I as described (*9*). By the same techniques, HTLV-II p24 was purified from a cell line (MC) developed in our laboratory. This cell line, which contains the complete integrated genome of HTLV-II, produces large quantities of virus. LAV was purified from supernatant fluids of primary lymphocyte cultures as described (*5*). Virus pellets were prepared from supernatant fluids obtained at the time of peak reverse transcriptase activity. The resuspended pellets were concentrated by centrifugation through a 30 percent sucrose cushion and then subjected to phosphocellulose chromatography. The product of this purification procedure was examined for homogeneity by electrophoresis on polyacrylamide gel.

We have developed a highly sensitive radioimmunoprecipitation assay that is specific for the major core protein, p25, of LAV. We used this assay, together with similar assays for antibodies to the core proteins (p24) of HTLV-I and HTLV-II, to determine antibody prevalence in groups of patients with AIDS on LAS, homosexual men, laboratory workers, and blood donors.

LAV was propagated in primary cultures of blood lymphocytes (*5*). At the time of peak reverse transcriptase (RT) activity, supernatant fluids were harvested from infected lymphocyte cultures by low-speed centrifugation, and virus was pelleted by ultracentrifugation

(100,000*g* for 2 hours). Virus-containing pellets were resuspended, centrifuged through a 30 percent sucrose cushion, disrupted with detergent, and subjected to phosphocellulose chromatography (*9*). HTLV-I p24 and HTLV-II p24 were similarly purified by phosphocellulose chromatography as described (*9*). The purified products were iodinated and examined by polyacrylamide gel electrophoresis (Fig. 1); the HTLV-I p24, HTLV-II p24, and LAV p25 were purified to apparent homogeneity. The immunoprecipitation of ^{125}I-labeled antigen by serum samples from patients was initially carried out at a 1:25 dilution. Samples were considered positive when at least 15 percent of the label was precipitated after the addition of fixed staphylococcal Protein A cells. Titers of several positive sera ranged from 1:50 to over 1:10,000. The specificity of the LAV p25 radioimmunoprecipitation assay was examined by a competitive radioimmunoassay (Fig. 2). Although unlabeled p25 from LAV competed well for homologous antibody (from patient B.R.U.), other retroviruses did not. The noncompeting viruses included HTLV-I, HTLV-II, Mason-Pfizer monkey virus, simian sarcoma virus, baboon endogenous virus, Rauscher murine leukemia virus, mouse mammary tumor virus, and equine infectious anemia virus.

Serum from AIDS patients was obtained either as part of various studies sponsored by the Centers for Disease Control (CDC) or as individual specimens submitted directly to CDC. Serum from LAS patients was obtained as part of an ongoing prospective study of this syndrome among homosexual men in Atlanta. Serum from CDC employees who worked in laboratories and other areas was obtained in 1983 and 1984; some of the laboratory workers were exposed to materials from AIDS patients. Serum samples were also collected from homosexual men, 18 years of age or older, who sought medical care at the San Francisco City Clinic. These samples were routinely collected from 1978 as part of ongoing studies of hepatitis B virus infection in homosexual men (*10*); specimens from 1984 were ob-

Table 1. Prevalence of antibodies to LAV p25 and the p24 of HTLV-I and HTLV-II in serum from AIDS and LAS patients and from control groups. Purified core proteins were labeled with ^{125}I and used as target antigens. Labeled antigen was added to serum samples and those samples that precipitated at least 15 percent of the label after the addition of staphylococcal Protein A were considered positive. N.D., not done.

Group	LAV p25			HTLV-I and HTLV-II p24		
	Number positive	Number tested	Percentage	Number positive	Number tested	Percentage
AIDS patients	51	125	41	7	125	5
LAS patients	81	113	72	0	113	
Controls						
Homosexual men						
1978	1	100	1	0	100	
1980	12	50	24	0	50	
Blood donors	0	189		0	N.D.	
Laboratory workers	0	70		0	70	

Table 2. Prevalence of antibodies to LAV p25 in serum from AIDS patients. All groups of AIDS patients are represented except hemophiliacs.

Group	Number positive	Number tested	Percentage
Homosexuals	42	100	42
Intravenous drug users	5	16	31
Haitians	2	5	40
Others	2	4	51
Total	51	125	41
Homosexuals by disease manifestation			
Kaposi's sarcoma	22	35	63
Opportunistic infection	29	90	33
Total	51	125	41

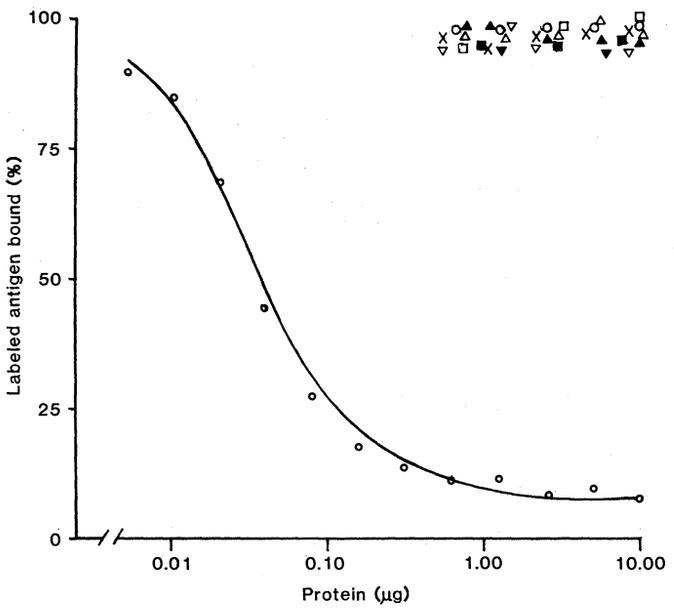

Fig. 2. Competitive RIA of the p25 of LAV and analogous core proteins of other retroviruses. The RIA's were carried out with ^{125}I-labeled LAV p25 and a limiting dilution of human serum positive for antibodies to LAV (B.R.U.). Serial dilutions (100 μl) of solubilized virus in buffer 1 [20 m*M* Na_2HPO_4 (*p*H 7.6), 200 m*M* NaCl, 1 m*M* EDTA, 0.3 percent Triton X-100, 0.1 m*M* phenylmethylsulfonyl fluoride, and bovine serum albumin (2 mg/ml)] were incubated with the appropriate serum for 1 hour at 37°C. Labeled LAV p25 (8000 count/min in 50 μl of buffer 1) was then added, and the mixture was further incubated at 37°C for 2 hours and at 4°C overnight. A 20-fold excess of goat antiserum to human immunoglobulin G was then added, and the volume made up to 1 ml in buffer 1. The samples were further incubated at 37°C for 1 hour and at 4°C for 2 hours and then centrifuged at 2500 rev/min for 5 minutes. The supernatant fluids were aspirated, and the radioactivity in the sediment was determined in a gamma counter. Virus extracts used for competition were as follows: ■, LAV; ▽, HTLV-I; ▼, HTLV-II; □, Mason-Pfizer monkey virus; X, simian sarcoma virus; and ○, equine infectious anemia virus.

tained, according to the same protocol, as part of a hepatitis B vaccine study (*11*). Serum from blood donors (provided by the National Red Cross) was chosen at random from samples obtained in 1980 and 1981. The donors were located in Tucson, Arizona; Madison, Wisconsin; and Philadelphia, Pennsylvania.

Specific antibody to the core protein of LAV p25 was present in serum from 41 percent of the AIDS patients and from 72 percent of the LAS patients (Table 1). In contrast, no antibody was detected in serum from the CDC employees or from the blood donors.

When we compared serum samples drawn in 1978 and 1984 from groups of homosexual men in San Francisco, we found a marked increase in antibody prevalence. In 1978, one of 100 homosexual men had antibody, and in 1984, 12 (24 percent) of 50 had antibody. Although the samples were collected and selected without known bias, to rule out confounding variables we are following the 1978 serum collection protocol exactly for the 1984 collection.

The prevalence of antibodies to LAV p25 varied only slightly among different groups of AIDS patients (Table 2). There was considerable variation, however, among AIDS patients with different disease manifestations. Patients with Kaposi's sarcoma alone had a significantly higher antibody prevalence [22 (63 percent) of 35] compared with patients who had only opportunistic infections [29 (34 percent) of 90] ($P = <0.005$, Student's *t*-test). These results are consistent with the observation that Kaposi's sarcoma patients are less immunodeficient than patients with opportunistic infections and support the contention that seropositivity decreases with disease progression.

Assays for antibodies to the p24's of HTLV-I and HTLV-II were conducted in parallel with the assays for LAV p25 antibody. The specificity of tests with these antigens has been described (*12*). Seven of the 125 AIDS patients had antibodies that precipitated the p24's of both HTLV-I and HTLV-II (Table 1). This concordance might be expected since the p24's of the two viruses are known to be cross-reactive (*12*). However, three of the same seven sera also precipitated the p25 of LAV—an unexpected result. Since neither HTLV-I p24 nor HTLV-II p24 competed in our LAV p25 competitive radioimmunoassay (RIA) (Fig. 2), and since p25 of LAV did not compete in complementary assays with homologous HTLV-I and HTLV-II p24 systems (*7*), this result must be explained otherwise. Some AIDS patients may have been infected with more than one virus, resulting in broadened specificity of antibody response. Other AIDS patients, infected with only one virus, may have developed a broader, more cross-reactive antibody against shared epitopes than has been found in the reference human and animal sera used in the development of the RIA. Support for possible shared common antigenic determinants among HTLV-I, HTLV-II, and LAV may come from nucleic acid hybridization studies in which varying degrees of homology between HTLV-I, HTLV-II, and LAV have been demonstrated (*13*). These studies indicate that LAV is more closely related to HTLV-II than to HTLV-I. LAV and HTLV-II cross-hybridize over most of their genomes, whereas LAV and HTLV-I cross-hybridize primarily in their *env* genes. This relationship may also account for the finding of antibodies in AIDS and LAS patients that react with those HTLV-I–encoded *env* gene products that are expressed on the plasma membranes of infected cells (*1*).

Thus we have shown that a high proportion of serum samples from patients with AIDS and LAS and from people at risk for AIDS has detectable antibodies to the major core protein of LAV. These antibodies also react specifically with LAV p25. Since other known type C and type D retroviruses do not cross-react in the competition RIA with ^{125}I-labeled p25 of LAV, these antibodies are not directed toward heterophile antigens that are commonly found in human serum (*14, 15*). The presence of antibody to LAV p25 in only 42 percent of the serum samples from AIDS patients may be due to the fact that these patients are highly immunosuppressed and have progressively lost the ability to make high-titer antibodies (*16, 17*). Another possible, but less likely, explanation may be that more than one virus is involved in the etiology of AIDS. A better indicator of LAV infection in these patients may be the presence of antibodies to the envelope glycoprotein of LAV. The antibody titers to the envelope glycoprotein may persist during the late stage of the disease while antibody to the core protein may decline to less than detectable levels. This has indeed been shown in the case of antibodies to the major core protein p24 and the presumptive envelope protein of HTLV-III (*8*).

Since we also find antibodies to LAV p25 in apparently normal homosexuals, the results of the study on the distribution of antibodies to LAV have to be interpreted with caution. For example, in the case of adult T-cell leukemia (ATL) in Japan, there is a clear etiological relation between the disease and HTLV-I (*15*). Even though nearly 25 percent of the population in the endemic area have antibodies to HTLV-I, only a minor percentage of the population gets ATL. Thus, as with ATL in Japan, other cofactors in addition to viruses may be involved in the causation of AIDS.

Note added in proof: A specific ELISA test with total LAV proteins detects LAV-specific antibodies in 95 percent of LAS patients and 70 to 95 percent of AIDS depending on the risk group and the stage of the disease.

V. S. KALYANARAMAN
C. D. CABRADILLA
J. P. GETCHELL
R. NARAYANAN
Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333
E. H. BRAFF
San Francisco City Clinic, San Francisco, California 94143
J.-C. CHERMANN
F. BARRÉ-SINOUSSI
L. MONTAGNIER
Département de Virologie, Institut Pasteur, 75724 Paris Cédex 15, France
T. J. SPIRA, J. KAPLAN
D. FISHBEIN, H. W. JAFFE
J. W. CURRAN, D. P. FRANCIS
Center for Infectious Diseases, Centers for Disease Control

References and Notes

1. M. Essex *et al.*, *Science* **220**, 859 (1983).
2. R. C. Gallo *et al.*, *Cancer Res.* **43**, 3892 (1983).
3. V. Hinuma *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6476 (1981).
4. M. Robert-Guroff *et al.*, *Lancet*, in press.
5. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983).
6. L. Montagnier *et al.*, in *The Cancer Cell 3*, R. C. Gallo and M. Essex, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).
7. P. M. Feorino *et al.*, *Science* **225**, 69 (1984).
8. M. Popovic *et al.*, *ibid.* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500; J. Schüpbach *et al.*, *ibid.*, p. 503; M. G. Sarngadharan *et al.*, *ibid.*, p. 506.
9. V. S. Kalyanaraman *et al.*, *J. Virol.* **38**, 906 (1981).
10. M. T. Schreeder *et al.*, *J. Infect. Dis.* **146**, 7 (1982).
11. D. P. Francis *et al.*, *Ann. Intern. Med.* **97**, 362 (1982).
12. V. S. Kalyanaraman *et al.*, *Science* **218**, 571 (1982).
13. R. Narayanan *et al.*, in preparation.
14. V. S. Kalyanaraman *et al.*, *Nature (London)* **294**, 271 (1981).
15. V. S. Kalyanaraman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1653 (1982).
16. H. C. Lane *et al.*, *N. Engl. J. Med.* **309**, 453 (1983).
17. E. Vilmer *et al.*, *Lancet* **1984-I**, 753 (1984); C. Cabradilla *et al.*, in preparation.
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