2d Draft - DS
3-1-85
(Lancet)

COMPARATIVE IMMUNOLOGICAL PROPERTIES OF LAV AND HTLV III

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INTRODUCTION

Two human retroviruses have been recently implicated as the causative agents of Acquired Immune Deficiency Syndrome (AIDS). The first virus described was designated Lymphadenopathy Associated Virus (LAV) since it was primary isolated from a homosexual male with lymphadenopathy syndrome (1) and then from AIDS patients (2)(3)(4)(5). Another human virus, named HTLV III has been also recently identified as a prime candidate for AIDS (6)(7). Several lines of evidences argue strongly that both viruses, LAV and HTLV III are similar and are indeed the primary cause of AIDS. They both show a preferential tropism for OKT_4^+ T lymphocytes (8)(22) and have cytopathic effects on this target T cells (2)(7). A high prevalence of antibodies to each of the viruses have been found in the sera of patients with AIDS or pre-AIDS (9)(10)(11)(12), and both viruses have been frequently isolated from all individuals at risk for the disease (2)(3)(4)(5)(6)(13).

Previous reports indicate that at least three major proteins, p25, p18, p13 are present in LAV virions (13)(14) and several studies indicate that p25 represents the major core protein (2)(15). A majority of AIDS or pre-AIDS patients have antibodies to LAVp25 (9)(10) but also to p18 and p13. A 45k protein has also been found but the viral origin of this component remains to be determined (14). Recently, a high molecular weight (gp110) has been identified to be the main antigenic component of the envelope protein (23).

Three major antigens p41, p24, p15 have been detected in HTLV III preparations (11)(12)(16) and the p24 has been considered as the major core protein whereas the p41 was assumed to be the envelope glycoprotein of HTLV III (11) (16). Althought all sera from AIDS patients contain antibodies to p41, some sera also react with the two lower molecular weight proteins (p24 and p15) (16).

Thus, LAV and HTLV III are retroviruses with similar structural and biological properties, but the relationship between these viruses has not yet been reported.

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In this paper, we present comparative data on the viral proteins and the antigenic correlation between LAV and HTLV III.

MATERIAL AND METHODS

VIRUSES

LAV was grown either on cord blood lymphocytes (2), on the LCo lymphoblastoïd B cell line (17) or on the T cell line CEM, and for the comparison with HTLV III, on the H_9 line. HTLV III was obtained from infected H_9 cell cultures, as previously described (7). Both viruses were concentrated by ultracentrifugation and purified by successive sedimentations through a 20 % sucrose cushion for HTLV III or a 30 percent sucrose cushion for LAV and a 20 - 60 % w/w sucrose gradient in TNE (10 mmol/l Tris-HCl, pH 7,4 containing 100 mmol/l NaCl and 1 mmol EDTA). In some experiments, the virus was only pelleted by high speed centrifugation without further purification.

COMPETITIVE RADIOIMMUNOASSAYS

Radioimmunoassays (RIAS) were performed by the double antibody method described earlier (10). Briefly, serial dilutions of solubilized virus, (initial concentrate 5 ug), used as competitive antigen, were preincubated for 1 hour at 37°C with a limiting dilution of a LAV1 positive patient serum (BRU) capable of precipitating 30 % of the labelled antigen.

Then, ^{125}I labelled LAV p25 (≃ 8000 cpm) was 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 furhter incubated 1 hour at 37°C followed by 2 hours at 4°C. The samples were centrifuged at 2500 cpm for 20 minutes and the percentage of radioactivity bound in the pellets was determined in an LKB ultragammacounter.

WESTERN BLOT ANALYSIS

Supernatants from noninfected H_9 cells or from viruses producing cell lines were ultracentrifuged overnight at 20000 rmp. The viral and control pellets (5-10 ug total protein) were lysed and fractionated by electrophoresis on SDS containing 12 percent polyacrylamide slab gel. The protein bands on the gel were electrophoretically transferred to nitrocellulose sheet according to the procedure of Towbin et al. (18). The sheet was incubated at room temperature for 1 hour in PBS supplemented with 1 % bovine serum albumine (BSA). The sheet was then cut into strips and each strip containing viral and control immunoblots was incubated for 1 hour at room temperature and overnight in cold in a screw cap tube containing PBS with 1 % BSA in which test sera (final dilution 1 : 250) were added. The strips were washed 3 times with PBS containing 0.3 % Tween 20, and incubated for one hour at room temperature with 1 % normal goat serum in PBS - Tween 20, washed with PBS - Tween 20. Then, goat anti-human IgG coupled with peroxydase was added for 1 hours at room temperature. The strips were washed with PBS-Tween 20, incubated with the substrate (diaminobenzidine tetrahydrochloride in PBS and H_2O_2) for 20 min. at room temperature, washed in distilled water and then dried.

INDIRECT IMMUNE FLUORESCENCE ASSAY

3×10^6 cells were washed with PBS and suspended in 200 ul of PBS. Aliquots (2-5 ul) were spotted on slides, air dried and fixed for 10 min. at room temperature in 50 % methanol : 50 % acetone. Slides were stored at -20°C until use. Ascites of hybridomas producing antibody against HTLV III p24 (BT_3) and HTLV III p15 (BT_2) or control ascites of p3 x 63 cells, diluted 1 : 200 in PBS were applied to cells and incubated 30 minutes. The fluorescein-conjugated $F(ab')_2$ fragment of sheep anti-mouse IgG (NL. Cappel Laboratories Inc. Cochvanville, pa) was purified through a human IgG-sepharose column, diluted and applied to cells. After 30 min. incubation at room temperature, slides were overnight in PBS before microscopic examination.

RESULTS

Several reports on the characteristics and the properties of either LAV (2)(13)(14) or HTLV III (6)(7)(11)(12) suggest that these viruses are probably the same or closely related viruses. As shown on Figure 1, by electron microscopy, LAV and HTLV III cannot be distinguished; they both show a characteristic morphology, distinc from HTLV 1 and HTLV II, with an eccentric small nucleoid in mature virions. The reverse transcriptase activity associated with LAV or with HTLV III shown a strong preference for Mg^{2+} and for the poly A oligo dT_{12-18} as template primer (7)(19) (and data not shown), a characteristic previously found in the other known human retroviruses, HTLV I and HTLV II (20).

Competitive radioimmunoassays were performed to determine the antigenic relatedness of LAV and HTLV III major core proteins. Purified LAV and HTLV III were used as competitive antigens in homologous radioimmunoassays for LAV p25. Figure 2, shows that HTLV III competes in the homologous LAVp25 assay. The quantitative pattern of this competition, as reflected by the shape and the slope of the curves, indicates that their major core proteins were identical.

In order to compare LAV proteins to HTLV III antigens, sera from French and American patients with AIDS or lymphadenopathy syndrome were examined by the sensitive Western Blot using either LAV or HTLV III as antigen preparations. Preliminary experiments using purified viral preparation confirmed the antigenic relationship between the major core protein of both viruses since four French sera containing antibodies to LAVp25 reacted with HTLV III p24, and conversely, three American sera containing antibodies to HTLV III p24 recognized LAVp25 (data not shown).

When concentrated viruses were used, the results presented in Figure 3 indicate that also other similar proteins were recognized by the same individual sera in each viral preparation. In this assay, the major antigens detected were the viral core protein p25 and a p34 protein. A 45K protein was also detected but the viral specificity of this antigen could not be determined since sera from normal subjects

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(Figure 3A) and from a patient who was positive for HTLV I (Figure 3E) reacted with this antigen. A reactivity with the protein of ea virus often appeared among the positive sera from AIDS or pre-AIDS patients. In each virus preparation, a high molecular weight prote (110 K daltons) was also recognized by some patients sera (Figure and 3G). Thus, this analysis indicates the immunological identity between LAV and HTLV III antigens.

These data were confirmed by indirect immunofluorescence assay as shown on Table 1, monoclonal antibodies to HTLV III p24 (21) or to HTLV III p15 (22) were able to label only HTLV III or LAV produc cells. However, the percentage of fluorescent cells in the positive sample varies. This can be explained by the high percentage of HTLV producing cells (80 %) in the H_9/HTLV-III cell line, as detected by immunofluorescence using rabbit antiserum to HTLV III or sera fr AIDS patients (7), comparatively to the lower level of viral expres sion by LAV infected permanent T cell line or B lymphoblastoid cell line. Only 1 to 20 % of LAV producing cells were positive by indirec immunofluorescence using patients sera (Table 1). Thus, the fluorescent labeling of LAV producing cell using specific monoclonal antibodies to HTLV III p24 or to HTLV III p15 confirms that HTLV III p24 is not the only antigen related to LAV, but HTLV III p15 is also similar to a low molecular weight antigen of LAV.

DISCUSSION

The data presented here show clearly that the major core protein of HTLV III is antigenically identical to LAVp25. Considering the fact that the internal virion core protein of retroviruses is a highly conserved interspecies determinant, these results indicate that LAV and HTLV III are either the same viruses or variants belonging to a same family of retroviruses. The fact that monoclonal antibody to HTLV III p15 recognizes LAV producing cells further support the homology between these viruses which has been already indicated by previously published data on the biological properties of these viruses (2)(13)(7)(8) and by seroepidemiological studies on their association with AIDS (9)(10)(11)(12).

In addition antibody reactions with selected sera from patien with AIDS or pre-AIDS show that these sera react with the same ant gens of HTLV III or LAV.

The major core protein p25 and a p34 antigen were predominant recognized by patients sera. Other antigens such as p18 and a hig molecular weight protein (110 K) were also detected in both viral preparations. Whether this high molecular weight polypeptide represent an envelope protein or an unprocessed polyprotein is under investigations.

Nevertheless, the pattern of antigenic-immune recognition presented here demonstrates that LAV and HTLV III are antigenically identical.

However, possible minor differences between each viral isolate may exist. Both viruses have been recently molecularly cloned. Molecular hybridization and nucleotide sequence have been investigate (F.Wong-Staal et al., accompanying paper) and suggest also that they are clearly related.

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ACKNOWLEDGEMENTS

We thank S. Chamaret, J. Gruest, D. Guetard and M.T. Nugeyre for technical assistance, C. Dauguet and C. Axler-Blin for electron microscopy examination and H.Sinno for secretarial assistance.

This work was supported by the Pasteur Institute, the CNRS and the French National League Against Cancer.

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Percentage of positive cells for LAV/HTLV III antigens detected by monoclonal antibodies or by patients sera.

CELLS	% positive cells		
	positive serum from pre-AIDS patient	monoclonal antibody to HTLV III p24	monoclonal antibody to HTLV III p15
H_9	0	0	0
H_9/HTLVIIIB	49	93	95
CEM	0	ND	ND
CEM/LAV	20	ND	ND
TiT_4	ND	0	0
TiT_4/LAV_I	ND	20	22
LCo/B LAV_I	2,5	1	1,3

Legend of Table 1

Indirect immunofluorescence assays were performed as indicated in details in Material and Methods.

When patient sera was used, it was diluted 1/10 in PBS before applied to cells, and the cells were stained with a fluoreseine conjugated antiserum to human immunoglobuline G.

Legend of Figure 1

Electron microscopy of thin sections of cells producing LAV or HTLV III :
(top) : LAV infected T lymphocytes
(bottom) : Hg cells producing HTLV III

Legend of Figure 2

Competition radioimmunoassay for LAV p25.
Radioimmunoassays were performed as described in Material and Methods using as competitive antigens either LAV (———) or HTLV III (-----).

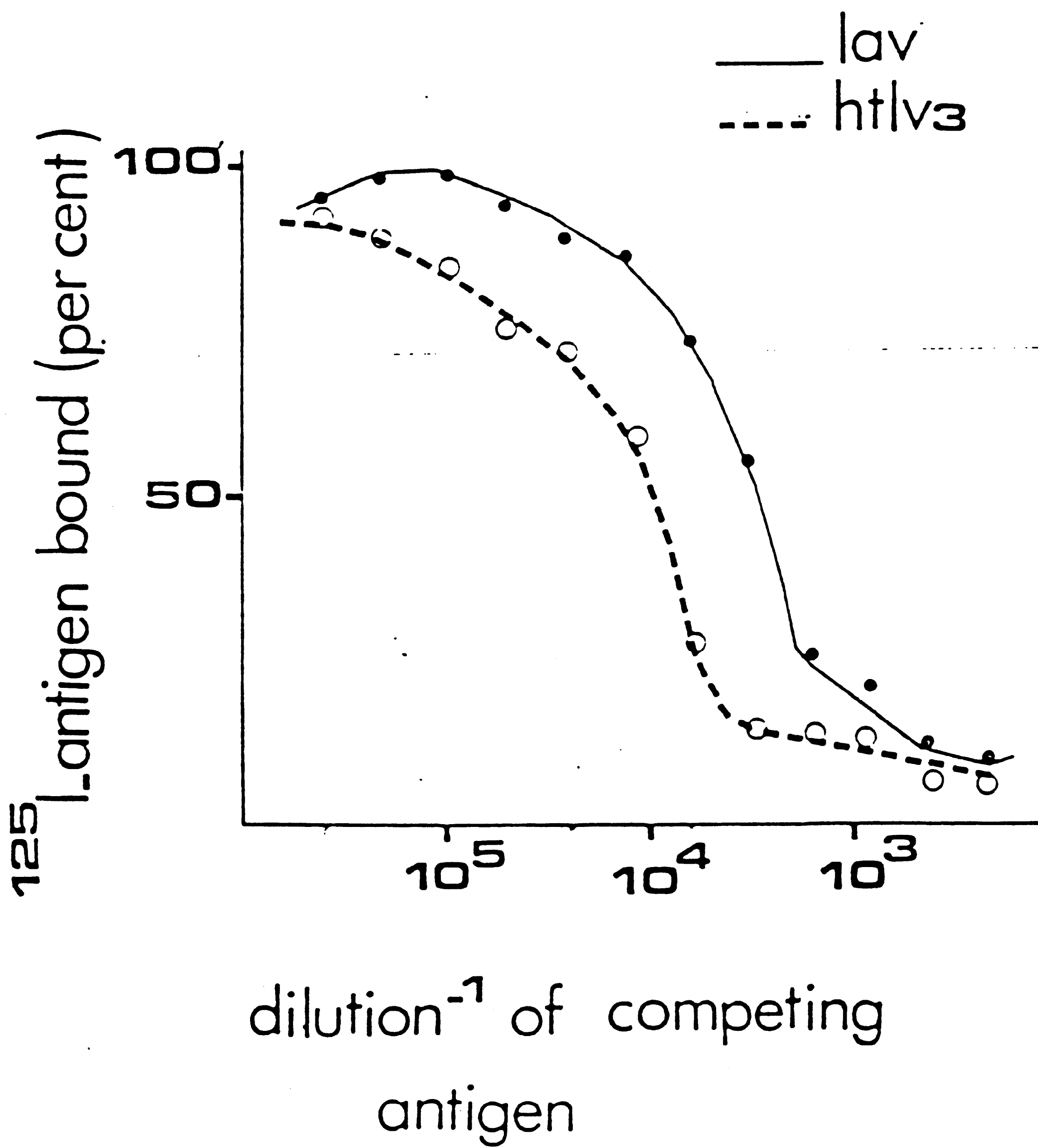
Legend of Figure 3

Comparison of LAV and HTLV III by Western Blot analysis.

The immunoblots were prepared as indicated in details in Material and Methods.

Each strips (A to D) contain viral and control preparations. Lane 1 : control obtained from the supernatant of non infected H_9 cell line; lane 2 : LAV from CEM producing cells; lane 3 : LAV from H_9 producing cells; lane 4 : HTLV III from H_9 producing cells.

Each strips were incubated with control or patients sera. Strip A : serum from a healthy individual; strip B: serum from a French homosexual with pre-AIDS; strip C: HTLV 1 positive serum; strip D: serum from a Haitian with AIDS, living in France.



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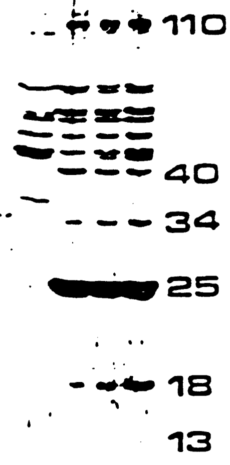
A

1 2 3 4

B

1 2 3 4

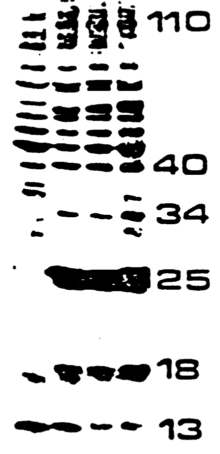


C

1 2 3 4

D

1 2 3 4



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