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Epidemiology of AIDS Virus and Characterization of Anti-AIDS Virus Antibody

Tsutomu MIYAMOTO, Hidenori SUGIYAMA, Shigeru KATAMINE,
Ryozo MORIUCHI and Shigeo HINO

Department of Bacteriology, Nagasaki University School of Medicine, Nagasaki 852, Japan

Introduction

It had been reported that human T-lymphotropic virus type 1 (HTLV-I) is cross-reactive with HTLV-III/Lymphadenopathy-associated virus (LAV)[1], causative agents of acquired immune deficiency symdrome (AIDS). Nagasaki Prefecture is known as one of the highest endemic area of HTLV-I in Japan and HTLV-I carrier rate in this area is 4 to 5% on an average[2]. Therefore, our first interest was whether HTLV-I infected individuals have also antibody to HTLV-III/LAV or not and we would like to confirm the cross-reactivity between two viruses.

During this investigation, we found a Japanese hemophiliac with anti-LAV/HTLV-III[5], and then we focussed our concern to examine Japanese hemophiliacs and to study seroepidemiology of LAV/HTLV-III in Japanese population. We report the results of these investigations and of characterization of anti-LAV/HTLV-III antibodies found in Japanese hemophiliacs.

Absence of anti-LAV antibody among adult T-cell leukemia (ATL) patients and carriers of HTLV-I

The virus and cells used are LAV and OKT4 positive cells, CEM, which were kindly supplied from Dr. Montagnier, Pasteur Institute in Paris. A control anti-LAV positive serum from a European AIDS patient was also obtained from Dr. Montagnier. Assay systems are indirect immunofluorescence (IF) and radioimmunoassay (RIA)[3].

As shown in Table 1, sera from ATL patients, HTLV-I carriers and control individuals (mainly healthy blood donors) had no detectable antibody to LAV. To the contrary, anti-LAV antibody gave no fluorescence on HTLV-I infected cells. This result was confirmed by radioimmunoprecipitation (RIP) (Fig. 2).

These results indicated that there was no possible cross-reaction between HTLV-I and LAV/HTLV-III[3].

Seroepidemiology of LAV/HTLV-III

In Table 2, the results of seroepidemiological studies by IF were summerized. 38 sera of hemophiliacs were obtained from University hospitals in Nagasaki, western part of Japan and

in Miyagi, north eastern Japan. 20 sera, about 53%, were seropositive for LAV. There was no detectable antibody to LAV in blood donors and chronic hemodialysis patients who tend to receive repeated transfusion. Therefore, the infection with LAV was concentrated upon hemophiliacs. In Japan, most, if not all, of factor VIII or IX products are imported from the United States. Taking this fact into consideration, it can be said that infection with LAV/HTLV-III in Japanese hemophiliacs is through imported factor XIII or IX. However, the dose dependency of used factor VIII or IX was not clear between seropositive and seronegative patients. And it is conceivable that although the number of materials are limited, LAV/HTLV-III does not yet penetrate into general population in Japan so far tested.

Characterization of anti-LAV antibody found in Japanese hemophiliacs

First of all, the specificity of anti-LAV antibodies found in Japanese hemophiliacs was examined by RIP. A representative result is shown in Fig. 1. Proteins with 18, 25, 40 and 55 Kirodultons(K) are gag gene products and glycoproteins with 43, 98 and 125K are env gene products. 18K protein was detected only when ^{3}H-leucine was used. 55K protein was thought to be precursor of 18 and 25K proteins and 125K glycoprotein might also be thought to be precursor of 43 and 98K glycoproteins. As seen in Fig. 1, the results of RIP by using two antibodies from European AIDS patient and Japanese hemophiliac were completely identical, indicating that anti-LAV antibody found in Japanese hemophiliac was specific for LAV proteins.

We compared the specificity of anti-LAV antibodies with anti-HTLV-I antibody in the next step. Left half of Fig. 2 is the result of RIP when HTLV-I infected MT2 cells[4] were used as antigen. The result when LAV were used as antigen is shown on right half of Fig. 2. The serum of HTLV-I carrier specifically immunoprecipitated gag gene products of HTLV-I, such as 24, 28 and 53K proteins and also env gene product, 62K glycoproteins when MT2 cells were used as antigen. However, sera from a European AIDS and a Japanese hemophiliac (HP-8) patients did not precipitate any HTLV-I proteins. Only the serum of HP-7 who is doubly infected with two viruses reacted to env gene product of HTLV-I. On the other hand, HTLV-I carrier's serum did not precipitate the LAV-specific proteins when using LAV as antigen. As described previously, immunological cross-reactivity between these two viruses was not recognized.

Fig. 3 shows the patterns of RIP of representative Japanese hemophiliacs' sera. H-15 precipitated p24 of HTLV-I which is differenciated from p25 of LAV. In the cases of Japanese hemophiliacs, the greater part immunoprecipitated the all of LAV-antigenic proteins. However, the smaller part such as HP-3 and -9 did only the glycoproteins. It became apparent that all 20 positive sera (Table 2) from hemophiliacs had the antibodies to LAV-glycoproteins, but 3(15%) lacked those to gag gene products of LAV.

As seen Fig. 3, there are no correlations between the IF antibody titer and the antibody titer against gag gene products, which are roughly estimated by radioactivity. For example,

IF antibody titer of HP-3 is 1:2560, but this serum did not contain any antibody to gag gene products. Therefore, we examined which LAV-proteins were adopted as antigens detected in IF, by antibody titration by RIP. Serially diluted HP-3 and HP-7 sera were applied to RIP. The results is shown in Fig. 4. Antibodies against env gene products, 98K and 125K could be detected up to 1:2560 dilutions in HP-3 and 1:640 dilutions in HP-7, respectively. These titers were well correlated with IF titers. Therefore, it was concluded that antigens detected by IF were mainly the glycoproteins of LAV.

False positive reaction in enzyme-linked immunosorbent assay (ELISA)

During seroepidemiological study of LAV, in addition of IF test, we carried out ELISA test for detecting anti-LAV/HTLV-III antibody by using a test kit certified by the United States and Japanese government.

The results are summarized in Table 3. Among 350 tested sera, 200 were from blood donors, 36 from ATL patients, 78 from hemodialysis patients and 36 from hemophiliacs. All of the IF-positive sera were also scored positive in ELISA. However, 4 sera, 1 of blood donor, 2 of hemodialysis patients and 1 of hemophiliac, were scored repeatedly positive only in the ELISA. Confirmation studies with RIP failed to demonstrate any anti-LAV/HTLV-III antibody activity, which indicated these 4 gave false positive reaction. Although the ELISA test is powerfull screening tool for detecting anti-LAV/HTLV-III antibody, false positive results as much as several percent might be expected. Therefore, it can be said that unless further confirmation tests, such as IF and RIP, are available, we must be thoroughly careful for adopting this test to general screening.

Conclusions

Infection of LAV/HTLV-III in Japan is concentrated upon hemophiliacs through the imported blood products, but does not penetrate into general population at present.

Antibodies against LAV/HTLV-III found in Japanese hemophiliacs and European AIDS patient were identical, indicating that the antibody was specific for LAV/HTLV-III proteins. And also it became apparant that HTLV-I was not cross-reactive with LAV/HTLV-III by confirmation with IF, RIA and RIP.

Some of sera from Japanese hemophiliacs lacked antibodies to gag gene products of LAV. Antibody titers to env gene products were well correlated with IF titers, indicating that antigens detected by IF were mainly the glycoproteins.

Finally, the ELISA test for detecting anti-LAV/HTLV-III showed false positive results as much as several percent. Therefore, the confirmation tests, such as IF and RIP, are necessary for adopting the ELISA test to general screening.

Table 1. Absence of anti-LAV antibody in HTLV-I carriers and ATL patients

	Anti-HTLV-I titers by IF		No. positive in		No. tested
	Range	Geometric Average	IF	RIA	
ATL patients	1/80-1/1280	1/340	0	0	36
HTLV-I carriers	1/10-1/2560	1/150	0	0	182
Control	-		0	0	284
Total			0	0	502

Table 2. Prevalence of anti-LAV antibody

	No. positive(%)	No. tested
Hemophiliacs		
Nagasaki	11(65)	17
Miyagi	9(43)	21
Blood donor	0	484
Hemodialysis patients	0	78

Table 3. Comparison of ELISA to IF for detecting anti-LAV/HTLV-III antibody

	No. IF-positive	No. IF-negative	Total
No. ELISA-positive	19	4	23
No. ELISA-negative	0	327	327
Total	19	331	350

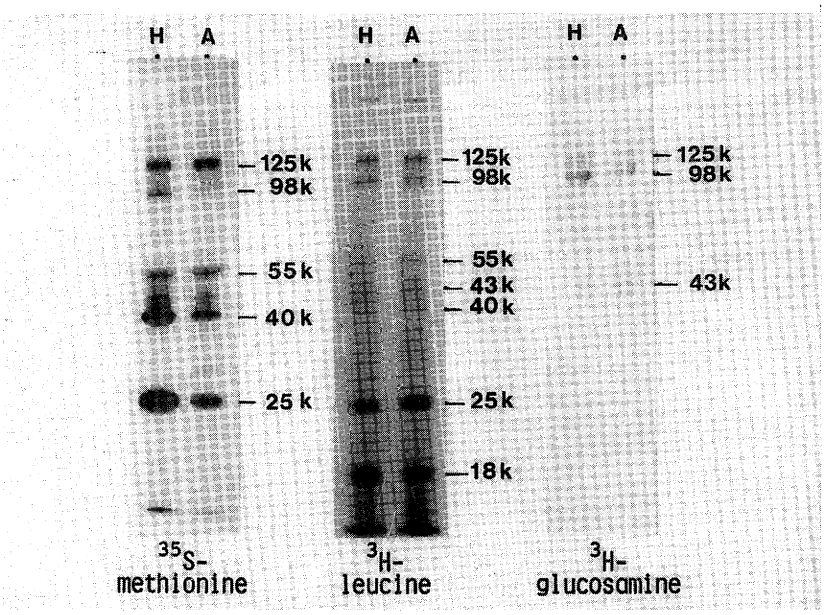

Fig. 1 Specificity of anti-LAV antibody found in a Japanese hemophiliacs.
H: Serum of a Japanese hemophiliac
A: Serum of a European AIDS patient

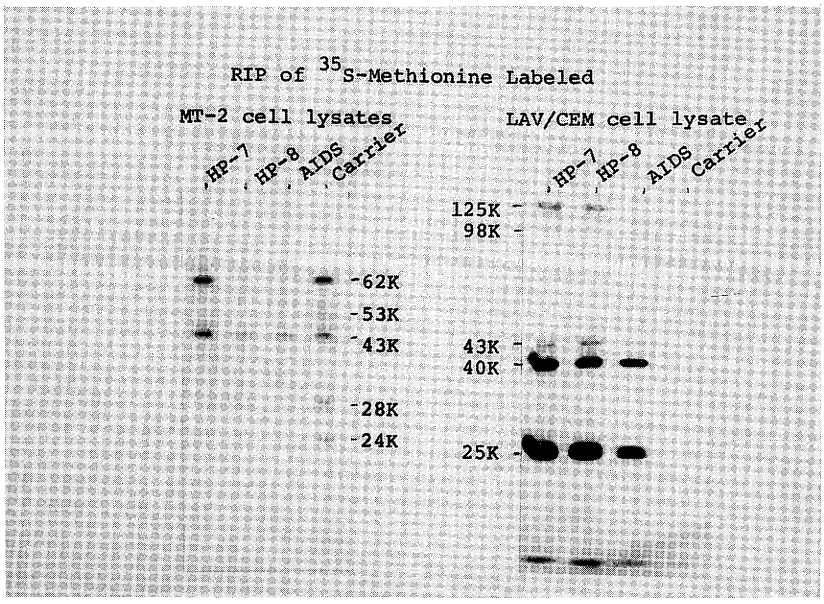

Fig. 2 Comparison of the specificity of anti-LAV antibody with anti-HTLV-I antibody.

AIDS	: Serum of a European AIDS patient
Carrier	: Serum of HTLV-I carrier
HP-7	: Serum of a Japanese hemophiliac doubly infected with LAV and HTLV-I
HP-8	: Serum of a Japanese hemophiliac with anti-LAV antibody

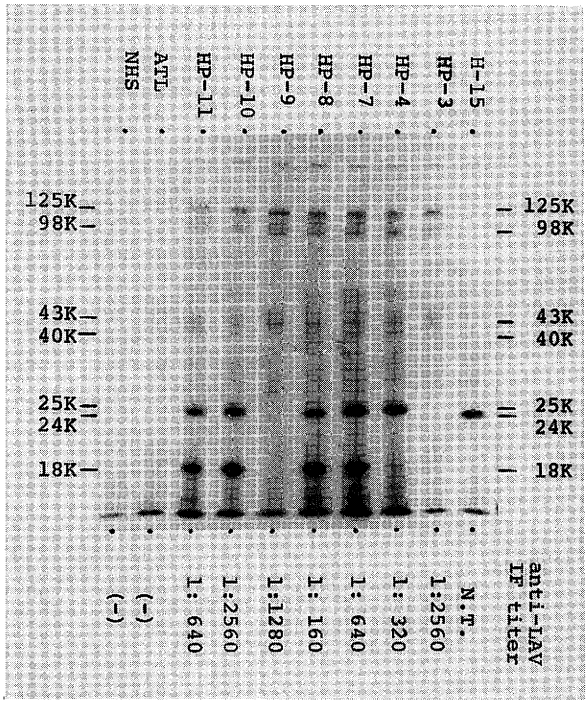

Fig. 3 Patterns of RIP of Japanese hemophiliacs' sera.
HP : Serum of Japanese hemophiliacs
NHS : Normal human serum
ATL : Serum of ATL patient
H-15 : Monoclonal antibody to p24 of HTLV-I
LAV/CEM was used as antigen except for lane H-15 in which MT-2 was used as HTLV-I antigen.

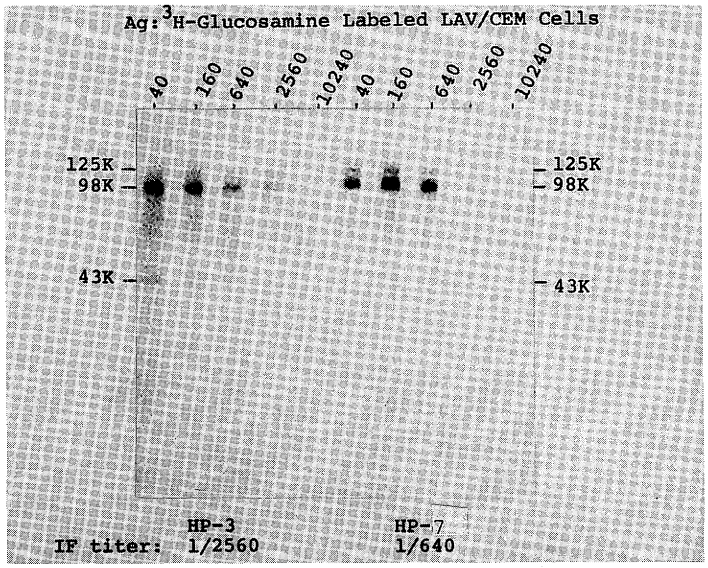

Fig. 4 The antibody titration by RIP
^{3}H-glucosamine labelled LAV/CEM cells were used as antigen.

References

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