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Prevalence of Human Immunodeficiency Virus (HIV) Infection in Japan

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In fall 1984, we started to conduct serological surveillance of HIV infection in Japan in collaboration with Dr. Hinuma, one of the chairmen. For the survey we employed indirect immunofluorescence and used TALL-1 cell infected with LAV as the antigen.[1] LAV was kindly supplied by Dr. Montagnier. In some cases we used ELISA and western blot. As already mentioned by Dr. Shiokawa and Dr. Abe, we have had more than 10 AIDS patients consisted of hemophiliacs and male homosexuals. We expected to have many HIV carriers in Japan. Besides, by now it is evident that many Asian countries have AIDS patients or HIV virus carriers including imported cases. Since we have not been able to develop medical strategies to prevent AIDS, it is urgent for us to minimize the spread of HIV among Japanese people. So far we have paid much attention to the status of HIV infections in the United States. And we got so many important informations. It is time for us to pay enough attention to all the foreign countries as well as inside Japan.

Figure 1 shows CCRF-CEM cells infected with LAV examined by scanning electron microscopy. In this electron micrograph, virus particles are coming out of the cell at a resticted area of the cell surface by budding process. So the progeny virions make a big cluster. In a higher magnification, we can see the cluster

of the virions clearer. If we observe the cells by immunofluorescence at this stage of infection, specific fluorescence can be seen as a crescent on the surface of the cell.[2] If we infect the cells at a higher input multiplicity of infection, we can get more number of viral antigen positive cells in early timing (data not shown). Figure 2 is the case of CEM infected by LAV at the MOI of 20, 2, 0.2 $TCID_{50}$/cell.

When we infect TALL-1 cells by LAV at the same input multiplicities of infection as CEM, we get less number of antigen positive cells after a long lag period. When we use TALL-1 cells infected by LAV as the antigen, the background is very low. The following data are the results employing this immunofluorescence system.

Figure 3 shows the results of antibody testing against LAV in sera of volunteer Japanese blood donors. Sera of 7,267 individuals were obtained from various parts of Japan. None of them showed positive result. Numbers in the parentheses represent the total number tested.

Sera of 30 patients who received multiple blood transfusions of more than 50 units in Kyushu University Hospital were kindly supplied by Dr. Okochi. They were tested for anti-LAV and anti-ATLA by immunofluorescence (Table 1). Sixteen out of 30 were positive for anti-ATLA but none of them were anti-LAV positive. This result reflects the fact that Kyushu Island is an endemic area of ATL. This result and the result shown in Figure 3 clearly show that HIV infection is not serious in the population of volunteer Japanese blood donors, so far.

Once contamination of hepatitis B virus vaccine with AIDS

virus was suspected. So we tested sera from children born to HBeAg positive mothers for anti-LAV. They received HBV vaccinations. None of them possessed anti-LAV in their sera(Table 2). Since the vaccine used in this study was produced from sera of Japanese HBV carriers, this fact again stressed the innocent situation of Japanese population in term of the contamination with HIV.

Table 3 is the situation of the supply of anti-hemophilic preparations in Japan in 1983. In summary, almost 80% of the preparations were made from American sources. The result of the use of these preparations will be shown in Table 4. Sera from 485 Japanese hemophiliacs were collected from various parts of Japan, from Hokkaido to Okinawa. They were tested for anti-LAV. One hundred and forty-one of them, i.e. 29.1%, were seropositive. Since there are 5,000 patients with hemophilia or related diseases in Japan, we expect more than 1,000 HIV carriers among them. In the course of our study, we realized that rates of seropositives among Japanese hemophiliacs varied from institutions to institutions or from place to place. For example, samples from Nagoya University showed always seropositive rate of around 20%, while in other cases, the ratios were more than 50%. This might be caused by the geographical difference of the sources for antihemophilic prepararions and not caused by the difference of test methods. In Figure 4, 4 cases (A,B,C and D) of Japanese hemophiliacs were added to the data presented by Eyster (1985).[3] On the top of the Figure, there are years when sera were collected. No.1 to No.30 are American cases and A, B, C and D are Japanese hemophiliacs. Open circle

represents seronegative, and closed circle seropositive. Although the number of cases is small, the data show that the timing of seroconversion in Japan had delayed comparing with that in the USA.

To confirm the results of immunofluorescence, sometimes we employed western blot assay[4]. We tend to assume that western blot is the best or the standard for anti-LAV tests. The result of western blot assay of sera of SLE patients, exhibited p24 band in 2 of 15 patients. In another case who did not have anti-LAV by IF test, western blot exhibited p17 and p24 bands. According to the report of American National Red Cross, out of 2,552 sera which were repeatedly reactive by ELISA, 10% were indeterminant by western blot. It can be concluded that we can not overestimate western blot and can not underestimate IF test.

Beside hemophiliacs, we have AIDS patients in Japanese male homosexuals, and, as already reported by Dr. Shiokawa, there are HIV carriers among Japanese male homosexuals. But the total number of the carriers is still small, maybe less than several thousands. We have got many informations from the experimence of HBV infection. But one thing, very much different from HBV infection, is that HIV has been imported from outside Japan and not endemic in Japan. So we have to pay much more attention and effort than HBV infections to minimize or erradicate HIV virus infection including screening of all donated blood units. At the same time, we have to establish strategies to prevent overt disease which will occur among HIV carriers in future. Such strategies may include the use of interferon or other drugs for virus carriers not for AIDS patients. This kind of medicares or

policies may improve the desperate situations of the carriers, especially after the notification of donors.

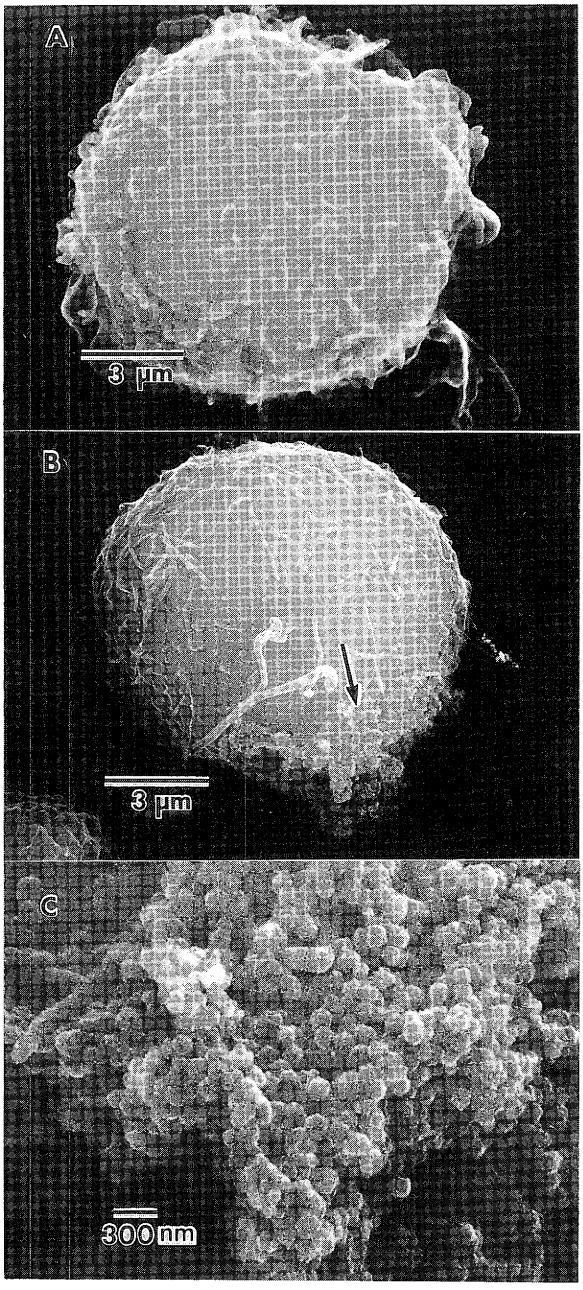

Fig. 1 Electron micrographs of CCRF-CEM infected with LAV.

A: non-infected cells

B: CCRF-CEM infected with LAV

C: higher magnification of a part of B indicated by the arrow

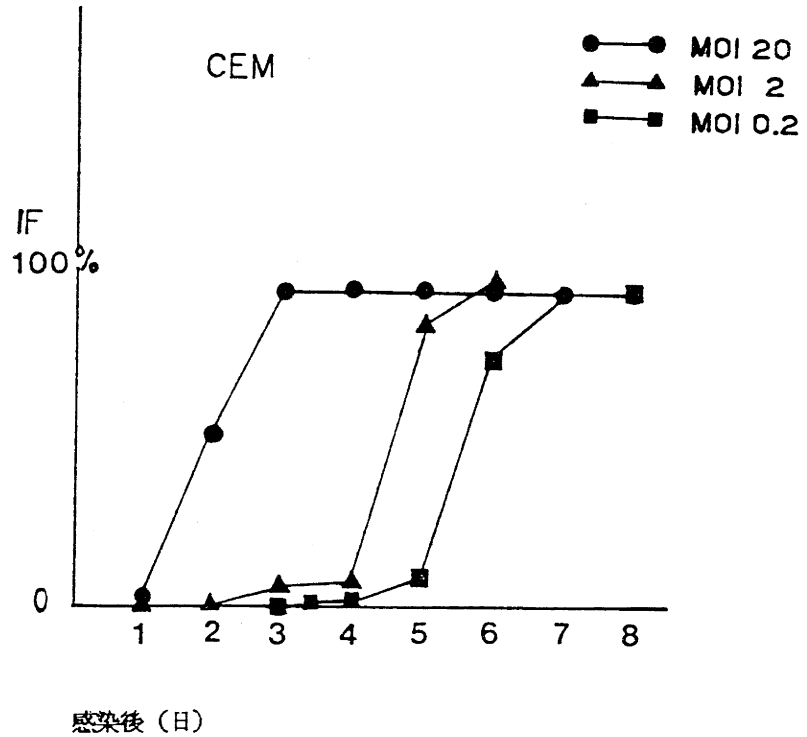

Fig. 2 Appearance of viral antigen positive CCRF-CEM after infection with LAV at various multiplicities of infection.

MOI (multiplicity of infection) is expressed by $TCID_{50}$/cell.

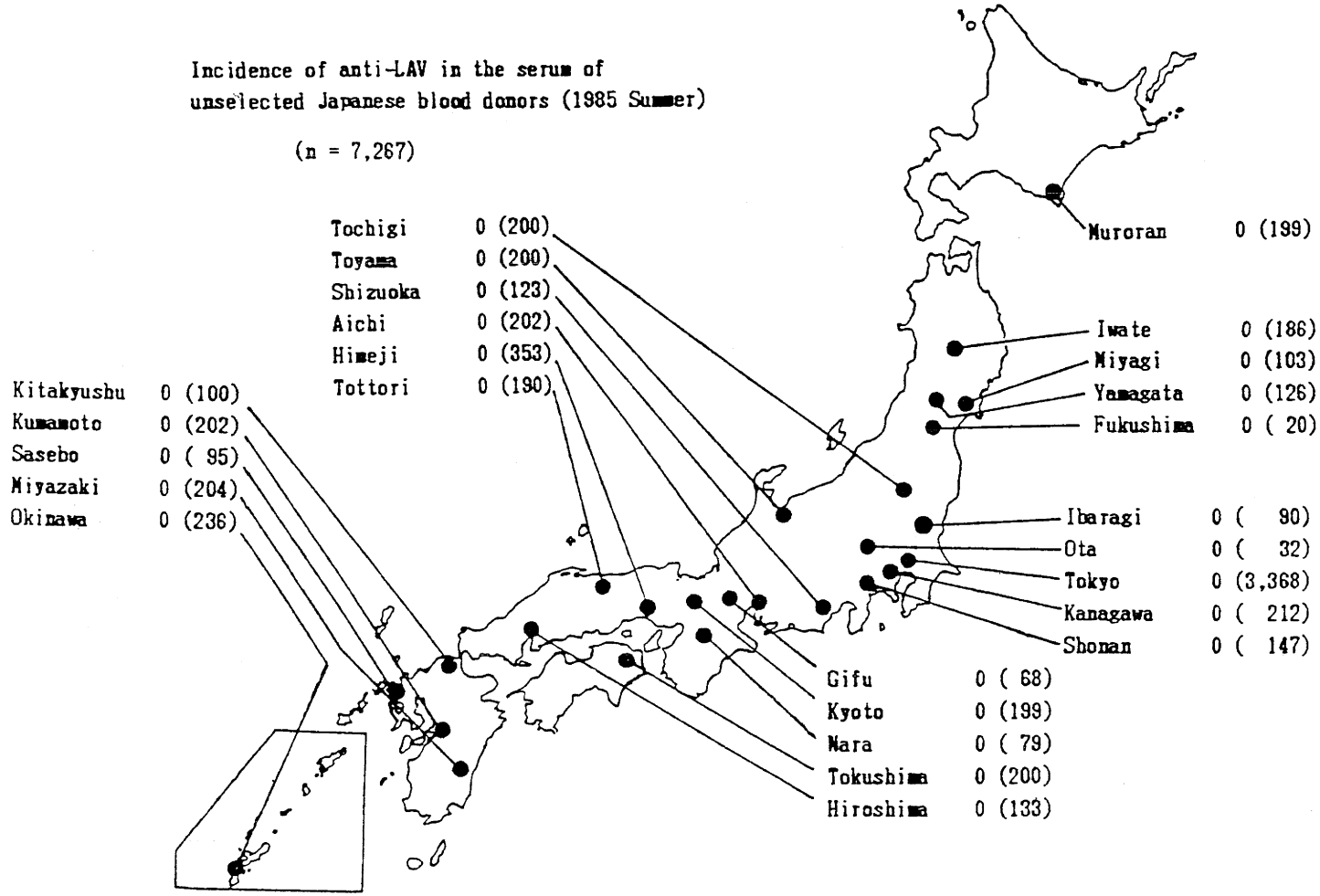

Fig. 3 Incidence of anti-LAV in the serum of unselected Japanese blood donors.

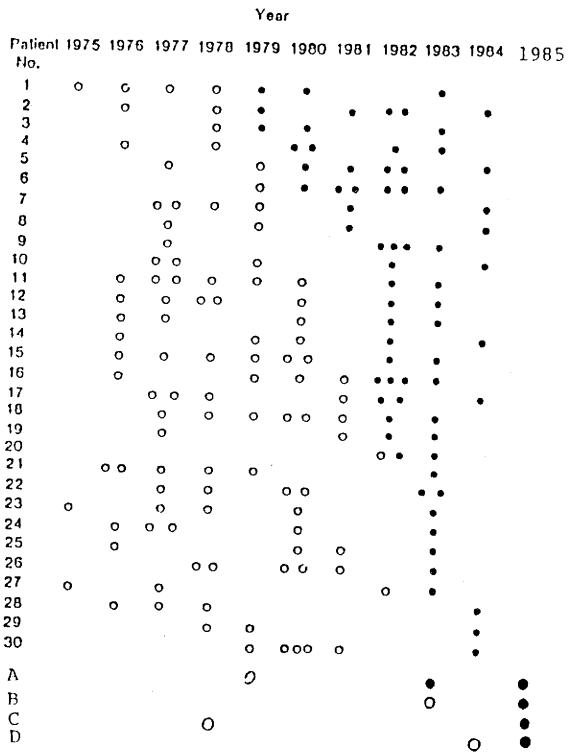

Fig. 4 Timing of seroconversion of hemophiliacs in the United States and in Japan.

1 - 30 : American patients. (Adopted from (3))

A - D : Japanese patients

o : antibody negative

● : antibody positive

Table 1. **Incidence of anti-LAV and anti-ATLA in serum of patients who received multiple blood transfusion of more than 50 units (Fukuoka, 1984)**

		anti-ATLA		total
		+	−	
anti-LAV	+	0	0	0
	−	16	14	30
total		16	13	30

Table 2. Prevalence of anti-LAV in children who received hepatitis B vaccine

Subject No.	a	b	c	n	schedule	anti-HBs	anti-LAV	anti-ATLA	
1	2	6.5	25	5	PPPAA	2^6	<5	<5	K
2	2	5	27	3	PPP	2^7	<5	<5	K
3	2.5	7.5	30	5	PPPPA	2^4	<5	<5	K
4	2	30	30	11	PPPPPAAAAAA	2^2	<5	<5	K
5	2.5	24	30	6	PPPPAA	2^5	<5	<5	K
6	2	8	19	4	AAAA	2^4	<5	<5	K
7	2	18	18	5	AAAAA	2^5	<5	<5	K
8	2	9	18	4	AAAA	2^4	<5	<5	K
9	2	3	18	2	AA	2^7	<5	<5	K
10	2	5	18	3	AAA	≧ 2^8	<5	<5	K
11	2	6	18	3	AAA	2^6	<5	<5	K
12	2	6	12	3	AAA	≧ 2^8	<5	<5	K
13	2	5	12	3	AAA	2^7	<5	<5	K
14	2	7.5	12	4	AAAA	2^3	<5	<5	K
15	2	5	12	3	AAA	2^8	<5	<5	K
16	1	4	10	3	AAA	2^6	<5	<5	K
17	2	5	12	3	AAA	2^5	<5	<5	K
18	2	5	16	3	AAA	2^7	<5	<5	K
19	2	10	11.5	5	AAAAA	2^3	<5	<5	K
20	3	6	25	3	AAA	2^7	<5	<5	K
21	2	5.5	18	3	AAA	2^8	<5	<5	K
22	2	7	12	4	AAAA	2^3	<5	<5	K
23	2	5.5	11	3	AAA	2^8	<5	<5	K
24	2.5	6	17	3	AAA	2^4	<5	<5	M
25	2.5	6	18	3	AAA	2^3	<5	<5	M
26	2	3	22	2	AA	2^5	<5	<5	M
27	2	5	18	3	AAA	2^6	<5	<5	M
28	2	5	24	3	AAA	2^6	<5	<5	M
29	2	7.5	12	4	AAAA	2^4	<5	<5	M
30	2	5.5	23	3	AAA	2^7	<5	<5	M
31	2	5	18	3	AAA	2^5	<5	<5	M
32	3	4	18	2	AA	2^8	<5	<5	M
33	2	5	17	3	AAA	2^7	<5	<5	M
34	2	5	12	3	AAA	≧ 2^8	<5	<5	M
35	2.5	5.5	13	3	AAA	2^8	<5	<5	M
36	2	8	15	4	AAAA	2^4	<5	<5	M

a: age in month when the first injection of HBV vaccine was performed.
b: age in month when the final injection of HBV vaccine was performed.
c: age in month when serum samples were taken.
n: number of HBV vaccine injections
P: plain vaccine
A: adjuvant vaccine
anti-HBs: measured by PHA
anti-LAV: measured by indirect immunofluorescence
anti-ATLA: measured by indirect immunofluorescence
K: Vaccine produced by Kitasato Institute
Protein content 32 μg/ml
plain vaccine 16 ~32 μg/one shot
adjuvant vaccine 8 ~16 μg/one shot
M: Vaccine produced by Green Cross 10 μg/0.5ml/one shot

Table 3. Antihemophilic Preparations Currently Used in Japan

Indication	Preparation	Company	domestic or imported
hemophilia A	Cryoprecipitate	Green Cross	domestic
		Nihon Seiyaku Co.	domestic
		Red Cross	domestic
	Factor Ⅷ concentrate	Green Cross	raw materials imported from the USA
		ALPHA-Green Cross	raw materials imported from the USA
		Nihon Seiyaku Co.	domestic
		Chemo-sero-therapeutic Research Institute	raw materials imported from the USA
		Travenol	prepared in the USA
		Travenol	prepared in the USA
		Cutter Japan	prepared in the USA
		Nippon Zoki	prepared in Austria
hemophilia B	Factor Ⅸ concentrate	Green Cross	prepared in Austria
		Nihon Seiyaku Co.	domestic
		Cutter Japan	prepared in the USA
		Travenol	prepared in the USA

Table 4. Anti-LAV in sera of Japanese hemophiliacs (1985.9)

Anti-LAV	+	141 (29.1%)
	−	344 (70.9%)
Total		485

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