

## Antibody to HTLV-I in Indigenous Inhabitants of the Andes and Amazon Regions in Colombia

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To explore the HTLV-I-carrying groups among the indigenous inhabitants in South America, a sero-epidemiological study on HTLV-I focusing on hinterland villages isolated from others in the Andes and Amazon regions was conducted. Five (2.9%) out of 171 subjects showed positive for HTLV-I antibody in the gelatin particle agglutination (PA) test. Two out of 5 positives with high antibody titer ( $\geq \times 1024$ ) in the PA test also showed a positive immunofluorescence (IF) test and anti-HTLV-I-specific protein products, p19, p24, p28, gp46, and p53 in sera by the Western blotting (WB) test. One of three negatives in the IF test showed positive antibodies to p19 and p24 by the WB test. Finally, two were confirmed as HTLV-I carriers and one was suspected of being a carrier. All three are Paez Indians from the central Andes; 53- and 34-year-old women and a 35-year-old man. The results show that HTLV-I carriers exist among isolated indigenous people in South America.

Key words: HTLV-I — South American Indians — Tropical spastic paraparesis — HTLV-I-associated myelopathy

Adult T-cell leukemia (ATL) was first reported in Japan<sup>1)</sup> and its causal retrovirus, human T-lymphotropic virus type I (HTLV-I), was isolated from ATL patients in the United States<sup>2)</sup> and independently in Japan.<sup>3)</sup> It is well known that patients with HTLV-I-associated diseases, i.e. ATL and tropical spastic paraparesis (TSP) or HTLV-I-associated myelopathy (HAM/TSP), are mainly distributed in Japanese in East Asia,<sup>1,4-6)</sup> and in blacks in Africa<sup>7,8)</sup> and the Caribbean basin.<sup>9-11)</sup> On the south Pacific coast of Colombia, most patients with HTLV-I-associated TSP were found amongst blacks of African ancestry.<sup>12)</sup> Furthermore, it was ascertained that HTLV-I carriers are clustered in some melanesians in Papua New Guinea.<sup>13-15)</sup>

From the paleo-anthropological viewpoint it was suggested that the first immigrants into South America arrived about 12,000 years ago from North Asia, passing through Beringia and North America,<sup>16)</sup> then gradually spread throughout the south American continent and settled mainly in the Andes and Amazon areas at least ten thousand years ago. On the other hand, the first immigration into the Japanese archipelago occurred more than several thousand years ago. One of the native Japanese "Ainu people" in Hokkaido possesses HTLV-I,<sup>17,18)</sup> and these people are regarded as being descendants of

the native population inhabiting Japan from the pre-agricultural Jomon period, more than 2,300 years ago. Both American Indians and Asians are grouped with the Mongolian people.

To establish the genetic and anthropological relationship between Asians and the indigenous inhabitants of South America (American Indians), it is important to clarify the existence of HTLV-I carriers among Indians in South America. Recently, several papers have reported that HTLV-I carriers might exist among the South American Indians and in people of mixed black and Caucasian ancestry.<sup>19-21)</sup> In an exploratory study on HTLV-I-associated diseases in South American Indians, three women with TSP/HAM from Paez village in the central Andes in South Colombia were found.<sup>22)</sup> Furthermore, one of us (V.Z.) found some American Indians from the Pacific coast of Colombia with serum antibodies to HTLV-I but not TSP/HAM (unpublished). These Indians live in Docordo on the San Juan river, near the Pacific coast but in the jungle. They are isolated from other villages and probably have not mixed with the blacks living in the coastal areas.

However, these previous studies did not approach the question of whether or not the Indians in South America have had a continuous reservoir of HTLV-I from ancient times or if they had been infected with HTLV-I from HTLV-I carriers among Japanese and/or blacks. To

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Fig. 1. A map of Colombia.

solve this question, we started a comparative epidemiological study of HTLV-I and its associated diseases, i.e. ATL and/or HAM/TSP, between Japanese in ATL endemic areas and Indians in the Andes and Amazon areas, in order to obtain information on some of the risk factors associated with the genetic background for manifestation of HTLV-I-associated diseases after infection with HTLV-I. As a first step, we conducted a sero-epidemiological study of HTLV-I among Indians in Southern Colombia.

To explore the clustering of HTLV-I carriers among native Indians in Colombia, we focused on the Andes and Amazon areas where many Indian groups isolated from others still live. We selected two areas for the present study and investigated five Indian groups; the Guambianos and Paez tribes in the Andes and the Ticunas, Yaguas and Witotos in the central Amazon region. As shown on the map (Fig. 1), Guambianos live in Silvia on the western side of the Andes near Popayan city, and the Paez people live on the eastern side of the central Andes located approximately five hours by jeep from the center of Popayan city. The Ticunas, Yaguas and Witotos live in small villages along the Amazon river

Table I. Positive Rate of Anti-HTLV-I Antibody among Indians in the Andes and Amazon Areas in Colombia (1989)

Location Town (group)	Number tested	PA+ (%) ( $\geq \times 16$ )	IF+ (%) ( $\geq \times 5$ )	WB+ (%)
Andes areas	85	3 (3.5)	2 (2.4)	2 (2.4)
Silvia (Guambiano)	53	0 (0)	0 (0)	0 (0)
Paez (Paez)	32	3 (9.4)	2 (6.3)	2 (6.3)
Amazon areas				
Leticia-Narino	86	2 (2.3)	0 (0)	0 (0)
(Ticuna)	47	2 (4.3)	0 (0)	0 (0)
(Yagua)	21	0 (0)	0 (0)	0 (0)
(Witoto)	15	0 (0)	0 (0)	0 (0)
(Others)	3	0 (0)	0 (0)	0 (0)
Total	171	5 (2.9)	2 (1.2)	2 (1.2)

PA, gelatin particle agglutination test (Serodia ATLA from Fujirebio); IF, indirect immunofluorescence assay on MT-1 cells; WB, Western immuno-blotting analysis (+: positive antibodies to p19, p24, p28, gp46 and p53).

around Leticia city and Puerto Narino town. These villages are isolated from their neighboring villages, and people in these villages might not have mingled with blacks on the Pacific coast.

During November 1-12 in 1989, we collected blood samples only from inhabitants recognized to be pure Indian; 85 samples from the Guambianos (53) and Paez (32) in the Andes areas and 86 samples from the Ticunas (47), Yaguas (21), Witotos (15) and others (3) from unknown groups in the Amazon (Table I). Each separated serum, sterilized by addition of 0.04% sodium azide was stored in a 2 cm<sup>3</sup> sample tube and taken to Japan. Antibody to HTLV-I in all samples was assayed at the Institute for Virus Research, Kyoto University. To screen the serum antibody to HTLV-I, we first employed the gelatin particle agglutination test (PA test: Serodia ATLA Fujirebio, Tokyo).<sup>23)</sup> Positive sera detected in the PA test (titer  $> \times 16$ ) were re-tested by immunofluorescence on MT-1 cells (IF test)<sup>3)</sup> and antibody specificity was confirmed by Western immuno-blotting analysis (WB test).<sup>24)</sup>

Five (2.9%) out of 171 subjects showed positive for HTLV-I antibody in the PA test (Table I). Three positives (9.4%) were from the 32 Paez people tested in the Andes area, and two (4.3%) were from the 47 Ticunas in the Amazon area. Two out of 5 positives showed high antibody titer ( $\times 1024$ ) in the PA test and also gave a positive IF test (titer  $\geq \times 5$ ), but the other 3 low-titer subjects were negative in the IF test (Table II). These two positives showed antibodies to HTLV-I-specific protein products, p19, p24, p28, gp46 and p53, in serum in the WB test. One of three negatives in the IF test showed

Table II. Positive Cases of Anti-HTLV-I Antibody by PA Test among Indians in the Andes and Amazon Areas in Colombia (1989)

Locality (region)	Number (initial)	Group	Age (sex)	PA (titer)	IF (>×%)	WB	Final judgement
Leticia (Amazon)	5 (A.L.C)	Ticuna	42 (female)	×128	—	—	Negative
	16 (M.R.)	Ticuna	28 (female)	×16	—	—	Negative
Paez (Andes)	21 (I.M.) <sup>a)</sup>	Paez	53 (female)	×1024	+	+	Positive
	24 (A.C.M.)	Paez	35 (male)	×32	NS	p19+ p24+	Not determined
	49 (F.C.) <sup>a)</sup>	Paez	35 (female)	×1024	+	+	Positive

PA, particle agglutination test (Serodia ATLA from Fujirebio); IF, indirect immunofluorescence assay by using MT-1 cells (NS: non specific and indeterminate); WB, Western blotting method (+: positive antibodies to p19, p24, p28, gp46 and p53).

a) With symptoms of TSP.

positive antibodies to p19 and p24 in the WB test. Finally, two subjects were confirmed to be carriers of HTLV-I and one was suspected of being a carrier of HTLV-I. All three are Paez Indians living in the Andes area; 53- and 34-year old women and a 35-year old man. The two women with high antibody titer had some clinical features of TSP/HAM.

The present sero-epidemiological study has identified two TSP/HAM cases and one suspected HTLV-I carrier among the Paez Indians. As shown on the map, Paez village is located in the hinterland of the central Andes far from the Pacific coast. It is interesting that HTLV-I carriers have been preserved only in a more isolated tribe, the Paez, but not in the other tribe, the Guambiano, in the Andes. From the incidence risk of HTLV-I associated diseases in Japan,<sup>25)</sup> We would expect that there are several hundred carriers of HTLV-I per patient with TSP/HAM or ATL. Several patients with TSP/HAM have been detected among the Paez Indians. It is assumed that more than 50,000 people live in Paez and its surrounding villages, so several hundred or more carriers of HTLV-I may live around Paez in the central Andes in Southern Colombia. Anthropologists have suggested that most of the Indian groups in the Andes immigrated from the Amazon, but there were no carriers of HTLV-I found among three Indian groups in the Amazon areas in the present study.

There are considerable numbers of HTLV-I carriers and patients with ATL or TSP/HAM among Colombian blacks<sup>12, 21)</sup> who originated from Africa and are mainly distributed in the coastal areas along the Pacific ocean and the Caribbean Sea. Many Indians but very few blacks live in the central Andes in Colombia. Since the 16th century, Spanish and other European people along with African blacks began immigrating into South America and settled mainly on the Pacific and Caribbean

coasts. Therefore, the ancestors of Indians settling down in the Andes should have had no chance to mix with HTLV-I carriers amongst the African black population before or after they immigrated into the Andes.

In the past 100 years, many Japanese, especially from southwestern Japan, have immigrated into the South American continent. However, they have settled in limited areas, e.g. Brazil and Bolivia. HTLV-I carriers have been detected among immigrant Japanese.<sup>20)</sup> There is a small society of Japanese immigrants near Cali in Colombia, but there is no evidence that they have moved into the hinterland villages of the Andes and mingled with Indians in those villages. Some HTLV-I carriers have been detected among mestizo<sup>12, 21)</sup> Indians of mixed Caucasian ancestry. There is some possibility that Spanish people infected with HTLV-I from blacks in the Pacific coast villages carried HTLV-I into the Indian population in the Andes areas. However, HTLV-I is mainly transmitted from mother to child, and is not highly contagious,<sup>26-29)</sup> so it should not have spread rapidly to the indigenous inhabitants in the hinterland Andes from blacks through the Spanish during the past several hundred years.

The preliminary results obtained from the present sero-epidemiological study suggest that HTLV-I carriers did exist among some indigenous South American Indians. To confirm this, a larger-scale sero-epidemiological study of anti-HTLV-I antibody in the Andes and Amazon, and HTLV-I isolation and genomic analysis are necessary. Furthermore, a serological study of human leukocyte antigens (HLA) in HTLV-I carriers and patients with HTLV-I associated diseases<sup>30)</sup> would give helpful information. We have already started a comparative HLA study between HTLV-I carriers in Japanese and in American Indians to clarify the differences and similarities of their genetic backgrounds.

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