Modes of transmission and evidence for viral latency from studies of human T-cell lymphotrophic virus type I in Japanese migrant populations in Hawaii

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ABSTRACT Human T-cell lymphotrophic virus type I (HTLV-I) seroprevalence was 20% among Hawaiian Japanese migrants (issei) and their offspring (nisei) from Okinawa compared to 35% in similarly aged men who were lifetime residents of Okinawa. A control group of migrants from a nonendemic area of Japan, Niigata, had low rates of HTLV-I antibodies, suggesting that Hawaii *per se* is not an endemic area for HTLV-I. Factors that were significantly associated with seropositivity in the Okinawa migrant groups were years of residence in Japan before migration (issei) and age for offspring of Okinawa migrants (nisei). Antibody titer was highest in Okinawa lifetime residents, intermediate in migrants (issei), and significantly lower in offspring of Okinawa migrants (nisei), with increasing titer observed with advancing age in the offspring of the migrant group. Based on these data, infection within the household occurring early in life appears to be a major route of HTLV-I transmission and may help to explain the curious geographic clustering of this virus in certain locales. As yet to be defined cofactors, including sexual transmission and/or environmental exposures (e.g., particularly before age 20), also may contribute to HTLV-I seropositivity. The pattern of rising seroprevalence and titer with age in the offspring of migrants who resided all of their lives in Hawaii raises the possibility that HTLV-I infection acquired early in life may become dormant and reexpressed with reactivation of latently infected T cells. The importance of this model in the process of viral leukemogenesis is supported by recent reports of adult T-cell leukemia in offspring (nisei) of Okinawa migrants.

Three classes of human T-cell lymphotropic retroviruses (HTLVs), one associated with lymphoid malignancy (type I; HTLV-I), one with acquired immunodeficiency syndrome, and one of uncertain disease relationship, have been discovered (1). HTLV-I clusters in circumscribed geographic locales in regions where the virus-associated adult T-cell leukemia/lymphoma, ATL, occurs (2). This sharp geographic localization of endemic virus infection suggests special circumstances that restrict viral infection outside of these locales. This pattern is perhaps most prominent in the Orient where the endemic occurrence of virus infection is restricted to a relatively small area. Thus, areas within the largest southern islands of Japan, Kyushu and Shikoku, and the islands of the Ryukyu archipelago including Okinawa, represent the highest rate areas in this region (3, 4). For unexplained reasons the virus is not prominent, even in the geographically proximal areas of Southern Honshu or in other populations in the region, including mainland China, Taiwan, Philippines, Vietnam, and Thailand (1).

The current study was undertaken to evaluate the occurrence of HTLV-I in migrant Japanese populations to Hawaii in comparison to lifetime residents of Japan. The results suggest that Hawaii is not an endemic area for HTLV-I but that high rates of infection are restricted to migrants and children of migrants from known viral endemic areas of Japan.

MATERIALS AND METHODS

From 1965 to 1968, there were 8006 men of Japanese ancestry born in the years 1900–1919 who participated in the Honolulu Heart Program on the Hawaiian island of Oahu (5). From 1967 to 1975, 7498 of these men returned for additional examinations. At that time, a nonfasting venous blood sample was obtained and stored at −20°C (1967–1970 specimens) or −75°C (1971–1975 specimens). Subsequently, this cohort has been followed prospectively as part of the Japan–Hawaii Cancer Study which has investigated cancer outcome and risk factors (6).

In this study, sera were identified from the following subjects: 98 issei men who were born in the HTLV-I endemic area of Okinawa prefecture (the Okinawa issei), mean age of 62.9 yr at time of phlebotomy; 107 nisei men who were born in Hawaii but whose parents came from Okinawa (the Okinawa nisei), mean age of 54.6; 20 issei men from the HTLV-I nonendemic area of Niigata (the Niigata issei), mean age of 65.7; 122 nisei men whose parents came from Niigata (the Niigata nisei), mean age of 56.1. These men were selected randomly from the cohort to represent their respective groups, except for the group of 20 issei men from Niigata, which included every issei subject from that prefecture. For comparison, 141 lifetime residents of Okinawa matched for age (50–70 yr, mean age 65.5) were collected from 40 male and 101 female residents of Okinawa prefecture as part of a diabetes mellitus survey. The 243 sera were from normal donors who were lifetime residents of Niigata, younger (mean age 26.8), and predominantly women (men, 10; women, 233) and thus are not exactly comparable to the remainder of the study groups but do provide information to confirm published data documenting the low prevalence of the virus in the Niigata population, including men at older ages (7, 8).

Sera were tested using the previously described whole-virus ELISA assay with confirmation of suspect samples (those with a ratio of test sample to control of >2) by competition assay as reported (9). Titers are expressed as the reciprocal of the serum dilution at which the absorbance of the test serum equaled the absorbance of a standard

negative serum diluted 1:20. A Z test based on a normal approximation to the two-sample binomial situation was used to compare proportions (10), and Spearman rank order or logistic regression were applied to correlations between population parameters and seropositivity (11, 12).

RESULTS

The prevalence of HTLV-I infection in the Okinawa population was higher than that of the Niigata study groups (Table 1). For the lifetime residents of Okinawa between the ages of 50 and 70, 15 of 40 men (37.5%) and 45 of 101 women (44.5%) were positive, documenting the fact that Okinawa has one of the highest rates of seropositivity among normal populations reported to date. The absence of positives in the Niigata residents confirms previous reports that this is an HTLV-I nonendemic area (7, 8). HTLV-I antibodies were rare in the Hawaiian–Niigata migrant groups, supporting the hypothesis that HTLV-I infection is not commonly acquired in the Hawaiian environment. On the contrary, Okinawa migrant groups to Hawaii reflected the high rate of seropositivity of lifetime Okinawa residents. Among the 98 Okinawa issei men, 20 (20.4%) were antibody positive, while 21 of 107 (19.6%) Okinawa nisei men were antibody positive. Although the overall rate of positivity in the Okinawa migrants (issei and nisei) (20%, 41 of 205) is significantly less than that of the male Okinawan lifetime residents (37.5%, 15 of 40; P = 0.008), these differences were not statistically significant when adjusted for age. The geometric mean antibody titer in the Okinawa lifetime residents was higher but not significantly different from that of the Okinawa issei men. The Okinawa lifetime residents had significantly higher titers, adjusted for age, than did the Okinawa nisei men (P = 0.0001). The titer, although not adjusted for age because of lack of age overlap, was significantly higher in Okinawa issei compared to nisei men (P = 0.016).

The age-specific prevalence of HTLV-I in the Okinawa issei and nisei men is contrasted with the pattern of positivity in Okinawa lifetime residents in Fig. 1 *Upper*. The rate in the Okinawa men and women was increased for all age groups compared to the Hawaiian cohort. Women have a slightly increased rate compared to men (P = 0.6) in the Okinawa residents, and there was no increase in seroprevalence with age, a pattern previously noted in populations in this older age group (3). For the nisei there was a significant correlation between age and antibody prevalence (r = 0.9, P = 0.042). This correlation was not significant for the issei (r = 0.8, P = 0.375). Titer did not vary significantly by age in the Okinawa residents or issei but rose with age in the nisei (r = 0.2; P = 0.036).

Among the Okinawa issei, the median age at which they moved to Hawaii was 15 and the maximum age was 20. As displayed in Fig. 1 *Lower*, there was a significant correlation (adjusted for age) between years in Okinawa before migration
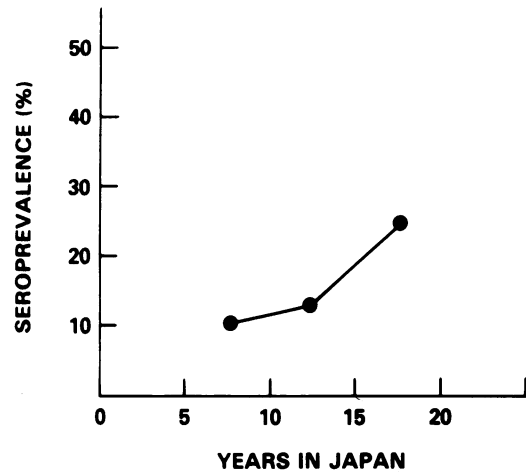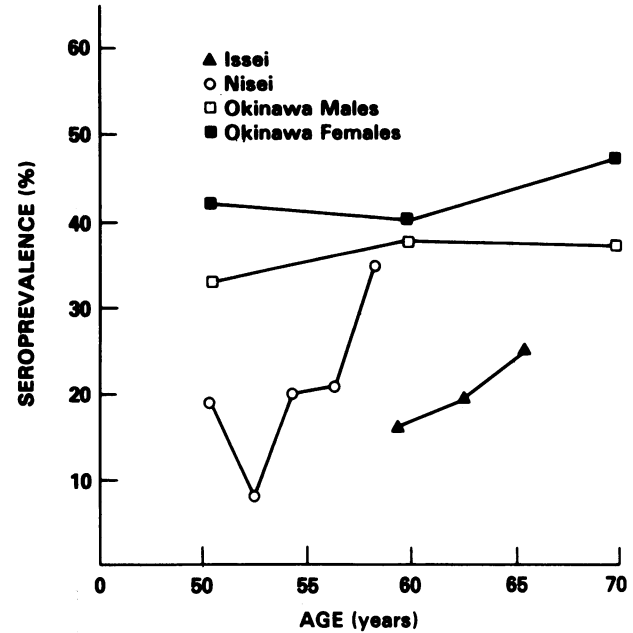

FIG. 1. (*Upper*) Age-specific prevalence of HTLV-I in sera (seroprevalence rates) of Okinawa lifetime residents and migrants to Hawaii. □, Okinawa men, n = 40; ■, Okinawa women, n = 101; ▲, issei, n = 98; ○, nisei, n = 107. (*Lower*) Seroprevalence rates in migrants from Okinawa to Hawaii (issei) by years of residence in Japan before migration to Hawaii.

and antibody prevalence (r = 0.16; P = 0.0316). Among the 107 nisei, 17 had returned to Japan for 1–9 yr (median, 6) and, of these, 3 (18%) were HTLV-I antibody positive. This rate of positivity is no different from the rate of 20% among the 90 Okinawa nisei who never left Hawaii (P = 0.4), and their titer values were no higher as well.

Table 1. HTLV-I seroprevalence in Japanese study subjects

	Okinawa*		Niigata*	
	No. +/No. tested	Geometric mean titer	No. +/No. tested	Geometric mean titer
Lifetime resident	15/40 (37.5%)[†]	50,272	0/243	NA
Issei	20/98 (20.4%)	31,171	1/20 (5%)	NA
Nisei	21/107 (19.6%)	8,721	2/122 (1.6%)	NA

All rates are for men age 50 yr or older, except for Niigata lifetime residents (median age, 27 yr) who were both male and female.
*Place of birth of study subjects. Numbers in parentheses are percentage of seropositivity; NA, not applicable.
[†]45/101 female lifetime residents of Okinawa were found to be positive from the same study group.

DISCUSSION

Epidemiologic analysis of the patterns of seroprevalence of HTLV-I antibody in normal populations in viral endemic areas has documented a curiously restricted geographic clustering of the virus and associated disease, a strong age dependence for seroprevalence, and a heightened rate of positivity in families with a pattern that suggests transmission within the household from husband to wife (sexual?) and mother to offspring (transplacental or perinatal?) (1, 2, 13, 14). In addition, some studies have suggested a role for environmental cofactors such as insect vectors, primarily because of the high antibody prevalence in association with certain vector-borne parasitic infections (15–18). The relative contribution of these suggested factors to the overall prevalence of virus has been difficult to sort out in viral endemic areas.

Results of the current analysis take advantage of the fact that migrant groups allow unique opportunities to control for factors in the environment that could be related to virus transmission. Thus, the data on the Niigata migrant groups indicate that Hawaii *per se* is not an HTLV-I viral endemic area. Both isseis and niseis from Niigata, where the prevalence of seropositivity to HTLV-I is negligible, continue to have a low HTLV-I antibody prevalence rate in Hawaii in contrast to migrants from Okinawa, where high rates persist. Based on these data, one can conclude that the rate of antibody positivity in migrant groups such as that from Okinawa is related primarily to exposures outside of the external Hawaiian environment.

The results of this analysis point to a strong role for household transmission. This is best seen in the Okinawa nisei group, where the level of antibody positivity was 19%. In this case there was no direct geographic contact with Okinawa, and for those 17 Okinawa nisei who did return to Japan, there was no increased risk of seropositivity. Thus, the group would appear to have sustained their exposure solely through infection in the household, possibly through maternal transmission. A role for mother to offspring transmission has been supported previously on epidemiologic grounds (13, 19) by the isolation of HTLV-I in the cord blood lymphocytes of a child born to an HTLV-I positive mother (20) and by experimental oral transmission of HTLV-I into marmosets (21). If the rate of positivity observed in this study population is at all representative of the efficiency of such transmission, it certainly suggests that this is a major mode of transmission. In this regard, the data provide useful information that help to explain the restricted distribution of the virus in the Orient. Thus, until recent times, the areas of Japan with high rates of positivity have been relatively isolated from the main population centers on the Japanese island of Honshu, although historically there has been substantial migration between Kyushu and Okinawa as part of various historic circumstances of feudal war and religious conflicts (22). Even within the endemic areas of Japan, regional clustering of virus positivity is associated with segments of the society that had limited outside contacts until recently (22–24). These data, although not providing direct evidence, do indicate that the virus could have been introduced into Kyushu from the outside and sustained in this tight environment by virtue of limited contact with the main islands of Japan until recent times (22).

In addition to household transmission, the patterns of seroprevalence in persons who actually lived in Okinawa versus those whose only contact with Okinawa was through their parents household suggest that circumstances in the Okinawa environment could contribute as well to the risk for seropositivity. First, the rate of positivity in lifetime Okinawa residents is up to 50% higher than that of the Okinawa issei or nisei matched for age. Although inhabitants of Okinawa experience one of the highest HTLV-I seropositivity rates in the world, it is unlikely that selective migration would account for the lower rates among Okinawans living in Hawaii (4). If anything, more of the migrants came from rural areas, where prevalence of HTLV-I antibody seems greater than in urban areas (23). Second, it was observed that the rate of positivity in the Okinawa issei group increased as a function of years in Okinawa prior to migration. Those who migrated to Hawaii in their late teens had a rate of positivity twice that of those who emigrated before age 12. A third observation was the differential in antibody titer, which was highest in lifetime Okinawa residents, intermediate in Okinawa issei, and lowest in Okinawa nisei. It is noteworthy that issei who left Okinawa in their teens had titers resembling those of lifetime residents while nisei who returned to Japan, primarily as young adults, for up to 9 yr had no difference in seroprevalence or antibody titer from those who never left Hawaii.

These data taken as a whole could suggest that living in Okinawa particularly from birth through adolescence may amplify one's risk for seropositivity to HTLV-I. Furthermore, titer, a measure of level of antigenic exposure, was also increased as a function of early life residence in Okinawa. It is unclear what circumstances explain this pattern. However, it is possible that environmental factors in Okinawa (e.g., hygenic practices or the presence of some environmental cofactor such as filariasis or strongyloides) account for this pattern. Since the population rate for seropositivity in Okinawa is higher than in Hawaii, perhaps the likelihood of exposure (e.g., sexual transmission in adolescence or household contact) explains this pattern. Finally, as discussed below, the level of antibody positivity could be influenced by the activation of latent retroviral infection by environmentally related antigenic stimulation (e.g., parasitic diseases or repeated HTLV-I reexposure).

One curious feature of the epidemiology of HTLV-I is the strong age dependence of the prevalence curve in all HTLV-I endemic areas studied to date (2, 3, 25). Rates of seropositivity are low in childhood, increase exponentially from adolescence, and plateau in the 50-yr-age group, usually with a slight female predominance. The pattern of positivity in the Okinawa lifetime cohort is consistent with the plateau phase of this curve (shown in Fig. 1 *Upper*). The age-dependent rise in seroprevalence has been previously ascribed to repeated exposure to the virus either through sexual contact or through environmental reexposure (1, 13). Thus, it was curious in this study that the Okinawa nisei had a significant age-dependent rise in antibody prevalence and titer, especially given the data described above for the Niigata group, which argue against the Hawaiian environment being a source for viral spread. One possibility is that female-to-male transmission accounts for this pattern although this mode has not been prominent for HTLV-I (13), and it is unlikely that sexual transmission would be more frequent in older nisei men, a conclusion supported by the observation that men in this age group residing in viral endemic areas do not show an age-dependent rise in seroprevalence after age 50. One novel possibility is that this pattern represents antibody response to a latent virus infection acquired early in life. Thus, latently infected T cells of antibody-negative persons could be recruited to proliferation and expression of viral antigens as part of the normal ongoing lifetime immune response. The titer data are consistent with this hypothesis as well. Although repeated viral exposures could account for these data, it is also possible that environmental antigenic exposures which amplify T-cell proliferation (e.g., parasitic infections), perhaps more prominent in Okinawa than in Hawaii, could explain this pattern. In this case, antibody would only be expressed when an infected T cell was recruited to sustained proliferation and antigen expression. In support of this

hypothesis are reports of virus-positive antibody-negative persons with HTLV-I and HTLV-III infection (26, 27) and animal models were latent retroviruses are linked to leukemia in the absence of viral antibodies depending on *in vivo* cell regulation (28).

In support of the hypothesis that HTLV-I acts as a leukemogen with a long latent period are the many cases of ATL developing in migrants from viral endemic areas who have moved to viral nonendemic areas (29, 30). The recent diagnosis of several ATL cases in Hawaii of persons of Okinawa ancestry, although not from this cohort, support this hypothesis (31). Of great interest is the fact that two of these cases were in nisei. This observation provides the first documentation that HTLV-I, presumably acquired perinatally, in an area away from environmental reexposure is linked to ATL. The age of these patients, both in their 60s, points to a long latent period from exposure to disease outcome.

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