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Antibodies to Human T-Cell Leukemia Virus-Membrane Antigens in Macaques with Malignant Lymphoma

P. J. Kanki[1,2], T. Homma[1], T. H. Lee[1], N. W. King Jr.[2], R. D. Hunt[2], and M. Essex[1]

A. Introduction

Gallo and co-workers first described human T-cell leukemia virus (HTLV), a type C retrovirus isolated from an aggressive case of cutaneous T-cell lymphoma [27, 28]. Cases of adult T-cell leukemia/lymphoma (ATLL), a unique mature T-cell malignancy, have also yielded numerous other isolates of HTLV [23, 7, 17, 35, 10, 29]. ATLL is seen at increased levels in southwestern Japan and the Caribbean, where seroepidemiologic studies have linked this tumor with HTLV type I. In such endemic regions, natural antibodies can be demonstrated in 4%–37% of the healthy adults whereas more than 90% of patients with ATLL have antibodies to HTLV (11, 31, 1, 32, 5, 20]. The prevalence of HTLV antibodies in healthy individuals is less than 1% in nonendemic areas [12, 6, 5, 20, 3, 4].

Miyoshi and his colleagues set the precedent for the nonhuman primate studies in 1982, when they demonstrated the presence of antibodies to HTLV in Japanese macaques (*Macaca fuscata*) by fixed-cell immunofluorescence [24]. Numerous studies since that time have identified antibodies to HTLV in other species of the genus *Macaca* as well as many African Old World primates. However, serological surveys by several investigators have not detected antibodies in New World primates or prosimians (lower primates) [24, 8, 14, 16, 34, 25, 9].

Macaque studies have shown rates of seropositivity ranging from 9% to 44% in healthy individuals [8, 14, 16, 34, 25, 9]. Familial clustering and an age dependence on the presence of HTLV antibodies have been demonstrated [25, 9]. Studies in Indonesia and Japan with wild-caught *Macaca fascicularis* and *Macaca fuscata,* respectively, show geographic clustering of seropositive individuals [8, 9, 25]. However, the geographic clustering of seropositive macaques in Japan does not appear to correlate to that seen in human populations [25, 9], suggesting that these closely related viruses have arisen independently. The studies to date have been conducted on apparently healthy macaques and there has been no disease or malignancy previously linked with this HTLV-related agent.

B. Methods

Serum samples from three species of captive macaques at the New England Regional Primate Research Center (NERPRC), Southborough, MA, were collected. In addition to sera from healthy macaques, serum samples from animals with the diagnosis of lymphoma or lymphoproliferative disorder (LPD) were included in this survey [13] (Table 1). These included: (a) *Macaca fascicularis,* the cynomologus macaque, 31 healthy controls and 5 with malignant lymphoma; (b) *Macaca mulatta,* the rhesus macaque, 30 healthy controls

1 Department of Cancer Biology, Harvard School of Public Health, Boston, MA
2 New England Regional Primate Research Center, Harvard Medical School, Southborough, MA

Table 1. Serum samples from three species of captive macaques at the NERPRC

	Diagnosis	Principle sites	Cell type
M. fascicularis M 10 yrs	ML	Mandible	Undifferentiated
M. fascicularis F 9+yrs	ML	Retro-orbital	Undifferentiated
M. fascicularis F 11 yrs	ML	Generalized	Lymphocytic
M. mulatta M 12 yrs	ML	Generalized	Lymphoblastic
M. mulatta F. 9 yrs	ML	Generalized	Lymphoblastic
M. mulatta F 10 yrs	ML	Intestine	Lymphoblastic
M. mulatta F 9+yrs	ML	Generalized	Histiocytic
M. mulatta F 4 yrs	ML	Generalized	Lymphoblastic
M. cyclopis F 4 yrs	LPD	Salivary gland, kidney, bone marrow	Lymphocytic
M. cyclopis F 4 yrs	LPD	Kidney	Lymphocytic
M. cyclopis F 3 yrs	LPD	Pancreas, kidney	Lymphocytic
M. cyclopis F 7 yrs	LPD	Bone marrow	Lymphocytic
M. mulatta F 4 yrs	LPD	Lung, salivary gland, kidney, bone marrow, bladder, muscle	Lymphocytic

M, male; F, female; ML, malignant lymphoma; LPD, lymphoproliferative disorder

Table 2. Antibodies to HTLV-MA in macaques

Health status	Cynomologus macaque *M. fascicularis*	Rhesus macaque *M. mulatta*	Taiwanese macaque *M. cyclopis*	Total
Healthy	2/30 (6.7%)	1/31 (3.2%)	2/14 (14.3%)	5/75 (6.7%)
Lymphoproliferative disorder	–	1/1 (100%)	3/4 (75%)	4/5 (80%)
Malignant lymphoma	3/3 (100%)	4/5 (80%)	–	7/8 (87.5%)

( ), percentage of seropositive macaques. Difference between healthy and malignant lymphoma/ lymphoproliferative disorder significant at $P < 9.42 \times 10^{-9}$, Fisher's exact statistic

and 5 with lymphoma and 1 with LPD; and (c) *Macaca cyclopis,* the Taiwanese macaque, 14 healthy controls and 4 cases of LPD.

Reports of spontaneous lymphoid malignancies in macaques are not common [26, 22]. There have been instances where multiple cases of lymphoma have occurred over a brief time interval, at least suggestive of a transmissible agent [15, 33, 19].

Malignant lymphoma and lymphoproliferative disorders have been observed in macaques at this colony over the past 12 years, and these malignancies are believed to be part of the macaque immunodeficiency syndrome previously described [15, 18]. A type change from C to D retrovirus has been isolated from macaques at this facility with this syndrome [2]; however, none of the macaques in this study were positive for the type D-New England virus. Complete autopsies were performed on all cases of lymphoma or LPD. Lymphoma cases varied in organ distribution, cell type, and degree of metastasis. LPD has been described, and is characterized by mature lymphocytic aggregates in the liver, pancreas kidney, and bone marrow [18].

The method for detecting antibodies to membrane antigens of HTLV-infected cells (HTLV-MA) has been described [3, 4, 20]. Antibodies to two glycoproteins, gp61 and gp45, encoded by the *env* gene of HTLV are detected in this assay. In addition, representative serum samples were subjected to radioimmunoprecipitation and sodium

Table 3. Presence of antibodies to HTLV-specific proteins[a]

Class of serum	*env*		*gag*	
	gp61	gp45	p24	p19
Human immunofluorescent antibody +, ATLL case	+	+	+	+
Human immunofluorescent antibody –	–	–	–	–
Macaque malignant lymphoma/lymphoproliferative disorder immunofluorescent antibody +	+	+	+	+
Macaque healthy immunofluorescent antibody +	+	+	+	+
Macaque healthy immunofluorescent antibody –	–	–	–	–

[a] Determined by radioimmunoprecipitation and SDS-PAGE of Hut 102 cell lysate

dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described earlier [20, 3, 4]. Whole-cell lysate of Hut 102 cells was used as the antigenic source since the major *env* and *gag* proteins have been established in this line.

C. Results

Table 2 gives the results of the indirect immunofluorescence assays in three species of macaques. Two of 30 healthy *M. fascicularis* had antibodies to HTLV-MA, whereas 3 of 3 macaques with malignant lymphoma were seropositive. Of 31 healthy *M. mulatta* only 1 macaque was seropositive in contrast to 4 of 5 with malignant lymphoma and 1 seropositive macaque with LPD. Of 14 healthy *M. cyclopis,* 2 macaques were seropositive whereas 3 of 4 with LPD had antibodies to HTLV-MA. In total, 11 of 13 macaques with malignant lymphoma or LPD were seropositive as compared with 5 of 75 healthy controls ($P < 9.42 \times 10^{-9}$, Fisher's exact test).

Table 3 indicates the HTLV-specific proteins immunoprecipitated by various sera analyzed. Human reference serum positive for antibody to HTLV-MA immunoprecipitates four major HTLV-specific proteins, with sizes of 19 000 (p19), 24 000 (p24), 45 000 (gp45), and 61 000 daltons (gp61), as has been described previously [20, 3, 4]. These proteins were not precipitated by human reference-negative serum. Immunofluorescent antibody-positive serum from a macaque with malignant lymphoma also immunoprecipitated the same HTLV-specific proteins. Similarly, an immunofluorescent antibody-positive serum from a healthy macaque precipitated the same size proteins. These same sera when subjected to lentil-lectin chromatography precipitated two proteins presumed to be gp45 and gp61, migrating at the expected position for the *env*-gene-encoded proteins, and these were not precipitated by immunofluorescent-negative macaque sera.

D. Discussion and Conclusions

Studies of the seroepidemiology and viral-related proteins of HTLV in humans conducted by our laboratory and others illustrate many common features with the non human primate studies. Macaque sera positive for antibodies to HTLV-MA recognize the same two HTLV-specific proteins, gp61 and gp45, most frequently recognized by human HTLV-MA-positive sera. Lee and co-workers have shown these to be surface glycoproteins and at least in part encoded by the *env* gene [20]. A high proportion of human antibodies reactive to HTLV-I-infected cells also react to HTLV-II C3-44/MO cells on membrane immunofluorescence and radioimmunoprecipitation. The HTLV-II gp67 is in part encoded by the *env* gene and comparison of the deduced NH_2 terminus amino acid sequences of type I and type II shows 65% homology in the first 40 amino acids. Thus, despite apparent divergence between two members of the HTLV family the major *env* gene

products show conservation in their deduced amino acid sequence and this is compatible with their serologic cross-reactivity [21]. It is therefore not unlikely that the simian member of the HTLV family might also show such conservation in its *env* gene products.

There is strong evidence that HTLV plays an etiologic role in its association with ATLL [6, 7, 35, 30]. Seroepidemiologic studies in people have shown that virtually all individuals with this tumor also have antibody to HTLV whereas the prevalence of seropositive healthy individuals is significantly lower. We have observed a similar pattern in the seroepidemiology of macaque lymphoma and lymphoproliferative disorder. Malignant lymphoma is not a common tumor of macaques; however, the incidence of this tumor at this particular colony has been high compared with non-lymphoid malignancies. Furthermore, successful transmission of malignant lymphoma from two of the macaques included in this study has been recently reported [15]. Our results indicate that an agent similar to HTLV type I is present in this colony, where it appears that the presence of antibodies to HTLV is associated with increased risk for the development of lymphoproliferative disorders or malignancies.

References

1. Blattner WA, Blayney DW, Robert-Guroff M, Sarngadharan MG, Sarin PS, Jaffe ES, Gallo RC (1983) Epidemiology of human T-cell leukemia/lymphoma virus. J Infect Dis 148:406–416
2. Daniel MD, King NW, Letvin NL, Hunt RD, Sehgal PK, Desrosiers RC (1984) A new type D retrovirus isolated from macaques with an immunodeficiency syndrome. Science 223: 602–605
3. Essex M, McLane MF, Lee TH, Falk L, Howe CWS, Mullins JI, Cabradilla C, Francis DP (1983) Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. Science 220:859–862
4. Essex M, McLane MF, Lee TH, Tachibana N, Mullins JI, Kreiss J, Kasper CK, Poon M-C, Landay A, Stein SF, Francis DP, Cabradilla C, Lawrence DN, Evatt BL (1983) Antibodies to human T-cell leukemia virus membrane antigens (HTLV-MA) in hemophiliacs. Science 221:1061–1064
5. Essex M, McLane MF, Tachibana N, Francis DP, Lee TH (1984) Seroepidemiology of human T-cell leukemia virus in relation to immunosuppression and the acquired immunodeficiency syndrome. In: Gallo RC, Essex M, Gross L (eds) Human T-cell leukemia viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 355–362
6. Gallo RC et al. (1982) Association of the human type C retrovirus with a subset of adult T-cell cancers. Cancer Res 43:3892–3899
7. Gallo RC, Mann D, Broder S, Ruscetti FW, Maeda M, Kalyanaraman VS, Robert-Guroff M, Reitz MS (1982) Human T-cell leukemia-lymphoma virus (HTLV) is in T- but not B-lymphocytes from a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 79:5680–5683
8. Hayami M, Ishikawa K, Komuro A, Kawamoto Y, Nozawa K, Yamamoto K, Ishida T, Hinuma Y (1983) ATLV antibody in cynomologus monkeys in the wild. Lancet 2:620
9. Hayami M, Komuro A, Nozawa K, Shotake T, Ishikawa K, Yamamoto K, Ishida T, Honjo S, Hinuma Y (1984) Prevalence of antibody to adult T-cell leukemia virus associated antigens (ATLA) in Japanese monkeys and other non-human primates. Int J Cancer 33:179–183
10. Haynes BF, Miller SE, Palker TJ, Moore JO, Dunn PH, Bolognesi DP, Metzgar RS (1983) Identification of human T-cell leukemia virus in a Japanese patient with adult T-cell leukemia and cutaneous lymphomatous vasculitis. Proc Natl Acad Sci USA 80:2054–2058
11. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita K-I, Shirakawa S, Miyoshi I (1981) Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci USA 78:6476–6480
12. Hinuma Y, Komoda H, Chosa T, Kondo T, Kohakura M, Takenaka T, Kikuchi M, Ichimaru M, Yunoki K, Sato I, Matsuo R, Takinuchi Y, Uchinao H, Hanaoka M (1982) Antibodies to adult T-cell leukemkia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide seroepidemiologic study (1982) Int J Cancer 29:631–635
13. Homma T, Kanki PJ, King NW, Hunt RD, O'Connell MJ, Letvin NL, Daniel MD, Desrosiers RC, Yang CS, Essex M (1984) Lymphoma in Macaques: association with virus of human T-lymphotrophic family. Science 225:716–718

14. Hunsmann G, Schneider J, Schmitt J, Yamamoto N (1983) Detection of serum antibodies to adult T-cell leukemia virus in non-human primates and in people from Africa. Int J Cancer 32:329–332
15. Hunt RD, Blake BJ, Chalifoux LV, Sehgal PK, King NW, Letvin NL (1983) Transmission of naturally occurring lymphoma in macaque monkeys. Proc Natl Acad Sci USA 80: 5085–5089
16. Ishida T, Yamamoto K, Kaneko R, Tokita E, Hinuma Y (1983) Seroepidemiological study of antibodies to adult T-cell leukemia virus-associated antigen (ATLA) in free-ranging Japanese monkeys (*Macaca fuscata*). Microbiol Immunol 27(3):297–301
17. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Blayney D, Golde D, Gallo RC (1982) A new sub-type of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571–573
18. King NW, Hunt RD, Letvin NL (1983) Histopathologic changes in macaque monkeys with an acquired immune deficiency syndrome (AIDS). Am J Pathol 113:382–388
19. Lapin B (1973) The epidemiologic and genetic aspects of leukemia among hamadrayas baboons of the Sukhumi monkey colony. In: Dutcher RM, Chieco-Bianchi L (eds) Unifying concepts of leukemia. Bibl Haematologica 39:263–268, Karger, Basel
20. Lee TH, Coligan JE, Homma T, McLane MF, Tachibana N, Essex M (1984) Human T-cell leukemia virus-associated membrane antigens: identity of the major antigens recognized after virus infection. Proc Natl Acad Sci USA 81:3856–3858
21. Lee TH, Coligan JE, McLane MF, Sodroski JG, Popovic M, Wong-Staal F, Gallo RC, Haseltine W, Essex M (1984) Serological cross-reactivity between envelope gene products of Type I and Type II human T-cell leukemia virus. Proc Natl Acad Sci USA (in press)
22. Manning JRS, Griesemer RA (1974) Spontaneous lymphoma of the non-human primate. Lab Anim Sci 24:204–210
23. Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraishi Y, Nagata K, Hinuma Y (1981) Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemia T-cells. Nature 294:770–771
24. Miyoshi I, Yoshimoto S, Fujishita M, Taguchi H, Kubonishi I, Niiya K, Minezawa M (1982) Natural adult T-cell leukaemia virus infection in Japanese monkeys. Lancet 2:658
25. Miyoshi I, Fujishita M, Taguchi H, Matsubayashi K, Miwa K, Tanioka Y (1983) Natural infection in non-human primates with adult T-cell leukemia virus or a closely related agent. Int J Cancer 32:333–336
26. O'Gara RW, Adamson RH (1972) Spontaneous and induced neoplasms in non-human Primates. In: Fiennes RNTW (ed) Pathology of simian Primates. Karger, Basel, pp 190–238
27. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 77:7415–7419
28. Poiesz BJ, Ruscetti FW, Reitz MW, Kalyanaraman VS, Gallo RC (1981) Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T-cell leukemia. Nature 294:268–271
29. Popovic M, Sarin PS, Robert-Guroff M, Kalyanaran VS, Mann D, Minowada J, Gallo RC (1983) Isolation and transmission of human retrovirus (human T-cell leukemia virus). Science 219:856–859
30. Reitz MS, Kalyanaraman VS, Robert-Guroff M, Popovic M, Sarngadharan MG, Sarin PS, Gallo RC (1983) Human T-cell leukemia/lymphoma virus: the retrovirus of adult T-cell leukemia/lymphoma. J Infect Dis 147:339–403
31. Robert-Guroff M, Nakao Y, Notake K, Ito Y, Sliski A, Gallo RC (1982) Natural antibodies to the human retrovirus, HTLV, in a cluster of Japanese patients with adult T-cell leukemia. Science 215:975–978
32. Schupbach J, Kalyanaraman VS, Sarngadharan MG, Blattner WA, Gallo RC (1983) Antibodies against three purified proteins of the human type-C retrovirus, human T-cell leukemia-lymphoma virus, in adult T-cell leukemia-lymphoma patients and healthy blacks from the Caribbean. Cancer Res 43:886–891
33. Stowell RC, Smith EK, Espana C, Nelson VG (1971) Outbreak of malignant lymphoma in rhesus monkeys. Lab Invest 25: 476–479
34. Yamamoto N, Hinuma Y, zur Hausen H, Schneider J, Hunsmann G (1983) African green monkeys are infected with adult T-cell leukemia virus or a closely related agent. Lancet 1:240–241
35. Yoshida M, Miyoshi I, Hinuma Y (1982) Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implications in the disease. Proc Natl Acad Sci USA 79:2031–2035