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Lymphoma in Macaques: Association with Virus of Human T Lymphotrophic Family

Abstract. *Human T-cell leukemia virus has been linked with adult T-cell leukemia-lymphoma (ATLL), a tumor of mature T cells that occurs at elevated rates in southwestern Japan and in the Caribbean Basin. Human T-cell leukemia virus (HTLV) or a closely related virus, has also been found in varying proportions of healthy individuals of several species of Old World monkeys. In the present study, conducted with macaques from Taiwan and the New England Regional Primate Research Center, antibodies to membrane antigens of HTLV-infected cells (HTLV-MA) were found in 11 of 13 macaques with malignant lymphoma or lymphoproliferative disease but in only 7 of 95 of healthy macaques. This indicates that antibodies to HTLV are significantly associated with the development of naturally occurring lymphoid neoplasms in at least some species of nonhuman primates.*

Human T-cell leukemia virus (HTLV) is a type C retrovirus that was first identified by Gallo and his colleagues in a patient with cutaneous T-cell lymphoma (*1*). Numerous isolates of HTLV were subsequently made from cases of adult T-cell types of leukemia-lymphoma (ATLL) from various parts of the world (*2*). Subsequent serological surveys for antibodies to HTLV have shown a strong association between HTLV and ATLL. Natural antibodies to HTLV have been demonstrated in more than 90 percent of the patients with ATLL as well as in 4 to 37 percent of the healthy adults in areas where ATLL is endemic (*3–7*). In areas where ATLL is not endemic, less than 1 percent of the healthy adults have antibodies (*4–8*).

Evidence that an agent similar to HTLV might be present in nonhuman primates was first reported in Japanese macaques (*Macaca fuscata*) by Miyoshi and his colleagues (*9*). This observation was further extended to indicate that many Asian and African species of Old World primates have antibodies to HTLV (*10–12*). Most extensively studied were species of the genus *Macaca*. Rates of seropositivity ranging from 9 to 44 percent have been reported in healthy macaques (*10–12*). The geographic distribution of seropositive macaques in Japan does not seem to be correlated with seropositivity in human populations (*11, 12*), suggesting an independent origin for the virus in humans and macaques. To our knowledge, there has been no evidence thus far linking lymphoma or other disease with the HTLV-related agent in nonhuman primates.

A seroepidemiological survey of macaques from Taiwan and the New England Regional Primate Research Center (NERPRC), Southborough, Massachusetts, was conducted. Included were sera from three species of healthy macaques and macaques diagnosed with malignant lymphoma (ML) or lymphoproliferative disease (LPD) at the NERPRC. Serum samples examined for antibodies were obtained from 95 healthy macaques and 13 macaques with ML or LPD. Of these, 20 were from healthy adult *M. cyclopis* captured and housed in Taipei, Taiwan. All other samples were from captive macaques at the NERPRC. Included among these were: 14 healthy *M. cyclopis* and 4 with LPD; 31 healthy *M. mulatta*, 5 with ML and 1 with LPD; and 30 healthy *M. fascicularis* and 3 with ML. Both LPD and ML have been described in conjunction with macaque immunodeficiency syndrome at the NERPRC. Lymphoproliferative disease is a lesion characterized by the presence of nodular aggregates of well-differentiated lymphocytes in the liver, kidney, or bone marrow (*13*); ML is the most common spontaneous neoplasm of macaques at the NERPRC observed over the past 12 years (*14*). All the ML cases were of the non-Hodgkin's type with variability in organ distribution, cellular morphology, and grade of malignancy. The diseased monkeys in this

Table 1. Presence of antibodies to HTLV-MA in macaques from Taiwan and the NERPRC. Antibodies to HTLV-MA were detected as described (*8*). Reference HTLV-infected cell lines, Hut 102 (*1*) and MT-2 (*20*) were harvested at the peak phase of logarithmic growth. One million cells were washed twice with phosphate-buffered saline (PBS) and reacted with 40 μl of a 1:4 dilution of serum at 37°C for 30 minutes. Preparations were then washed twice with PBS and exposed to 20 μl of a 1:20 dilution of fluorescein-conjugated immunoglobulin G fraction of goat antiserum to monkey immunoglobulin G (Cappel, Cochranville, Pennsylvania). The samples were incubated at 37°C for 30 minutes, washed twice with PBS, and examined for fluorescence. Samples were considered positive when more than 40 percent of the target cells showed fluorescence. Positive and negative human reference sera were included in each test. Sera that initially scored positive were tested on two uninfected human lymphoid cell lines, 8402, a T-cell line (*21*), and NC 37, a B-cell line which lacks surface immunoglobulin in (*22*), to confirm specificity.

Species	Clinical status	Origin	Number tested	Number positive	Percent positive
M. cyclopis	Healthy	Taiwan	20	2	10.0
	Healthy	NERPRC	14	2	14.3
	LPD	NERPRC	4	3	75.0
M. mulatta	Healthy	NERPRC	31	1	3.2
	LPD	NERPRC	1	1	100.0
	ML	NERPRC	5	4	80.0
M. fascicularis	Healthy	NERPRC	30	2	6.7
	ML	NERPRC	3	3	100.0
Total*	Healthy		95	7	7.4
	ML/LPD		13	11	84.6

*Difference between healthy and ML or LPD significant at $P < 6 \times 10^{-9}$ with Fisher's exact test.

study were the same animals whose pathology was described earlier (*13*).

Sera were examined for antibodies to membrane antigens of HTLV-infected cells (HTLV-MA) as described (*8*). This procedure detects antibodies to two glycoproteins expressed on HTLV-infected cells that are encoded by the *env* gene of HTLV (*7*). Sera were tested on two reference HTLV-I–infected cell lines, Hut 102 and MT-2. Those that scored positive were tested on uninfected T (8402) and B (NC 37) lymphoid cell line to exclude false positives as described earlier (*7, 8*). Antibodies to HTLV-MA were identified in four of 34 healthy *M. cyclopis* (Table 1). Two of the seropositive macaques were from Taiwan, and the other two were from the healthy population at the NERPRC. In contrast, three of four *M. cyclopis* with LPD were positive for antibodies to HTLV-MA. Of 31 healthy *M. mulatta* from the NERPRC only one was seropositive. Five of six *M. mulatta* with ML or LPD had HTLV-MA antibodies. In *M. fascicularis*, two of 30 healthy individuals had HTLV-MA antibodies while all three with ML were positive. Overall, 11 of 13 (84.6 percent) macaques with ML or LPD had antibodies to HTLV-MA whereas seven of 95 (7.4 percent) of the healthy controls were seropositive. The results obtained on Hut 102 and MT-2 were similar. None of the positive sera reacted with the uninfected cells. Macaques with ML or LPD had antibodies to HTLV-MA significantly more often than healthy macaques ($P < 6.27 \times 10^{-9}$; Fisher's exact test).

Representative serum samples were subjected to radioimmunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to analyze the reactivity of the antibodies for known HTLV proteins. Sera were reacted with whole cell lysate of Hut 102 cells since the major *gag* and *env* proteins have been defined in this line (Fig. 1A). A human reference serum positive for antibodies to HTLV-MA and HTLV proteins precipitated proteins with sizes of 24,000 daltons (p24), 45,000 daltons (gp45), and 61,000 daltons (gp61), as described (*7, 8*) (see Fig. 1A). Sera from healthy *M. cyclopis*, one from Taiwan and one from the NERPRC, recognized the same proteins. A serum sample from a *M. fascicularis* with ML showed the same reactivity (Fig. 1A). The same sera were also reacted with glycoproteins prepared from Hut 102 cells by using lentil-lectin affinity chromatography (Fig. 1B). Two proteins presumed to be gp45 and gp61, which migrated at the positions expected for the *env* gene encoded proteins, were precipitated with sera positive for HTLV-MA antibodies but not with negative sera (Fig. 1B).

Our results extend previous observations that infections with an HTLV-related agent occur in macaques. Of 20 *M. cyclopis* captured and housed in their natural habitat, Taiwan, 10 percent had antibodies to HTLV-MA. The rate of seropositivity among healthy *M. cyclopis* raised in captivity at the NERPRC was similar. Of greater interest, however, is the correlation between exposure to HTLV and the presence of ML or LPD in macaques. Spontaneous ML has been observed in macaques during the last 12 years at the NERPRC, and successful transmission of ML was reported (*14*). An LPD has been described in animals with the macaque immunodeficiency syndrome described at the NERPRC. Macaques that developed either of these spontaneous lymphoproliferative abnormalities had higher rates of exposure to HTLV than healthy controls. Earlier reports have described infections with a type D retrovirus in macaques that develop immunodeficiency syndrome (*15*). However, antibodies directed to the HTLV gp61 and gp45 should not cross-react with the type D retroviruses.

The association between HTLV and

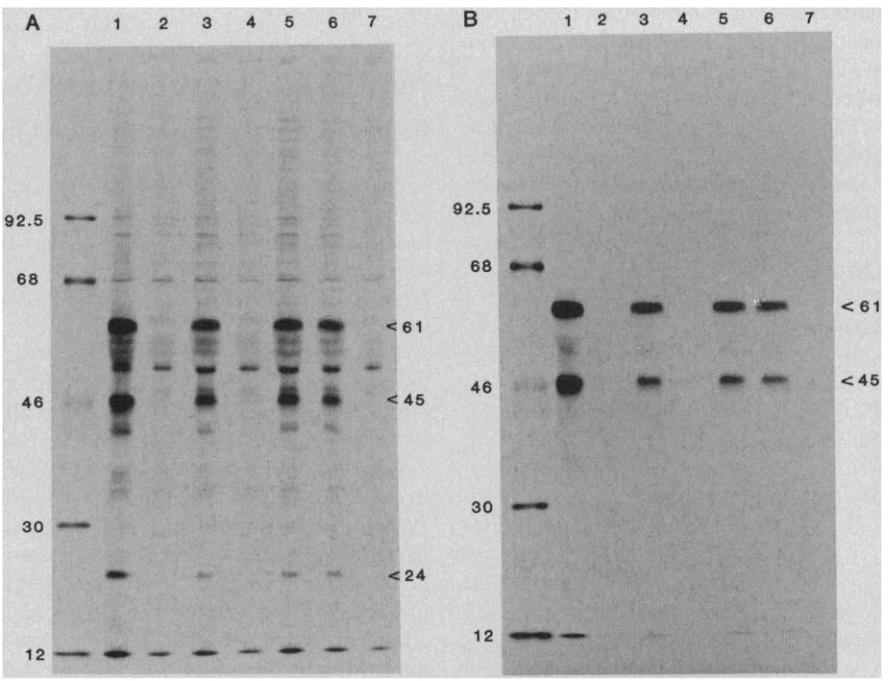

Fig. 1. (A) Reactivity of serum samples from macaques positive for antibodies to HTLV-MA was determined by radioimmunoprecipitation in conjunction with SDS-PAGE as described (*7, 8*). Hut 102 cells were harvested at their peak of log-phase growth and were exposed to [^{35}S]cysteine [100 μCi/ml; specific activity 1000 to 1050 Ci/mmol; New England Nuclear (NEN)] for 8 to 10 hours. A soluble cell lysate was prepared by disrupting cells with RIPA buffer (0.15*M* NaCl, 0.05*M* tris-HCl, *p*H 7.2, 1 percent Triton X-100, 1 percent sodium deoxycholate, and 0.1 percent SDS) and clearing by centrifugation for 1 hour at 100,000*g*. The cell lysate was reacted with 10 μl of the following test sera bound to Protein A–Sepharose CL-4B (Protein A beads, Pharmacia, Sweden): (Lane 1) A human reference serum positive for antibodies to HTLV-MA; (lane 2) a human serum negative for antibodies to HTLV-MA; (lane 3) serum from a healthy adult *M. cyclopis* from Taiwan that is positive for antibodies to HTLV-MA; (lane 4) serum from a representative healthy adult *M. cyclopis* from Taiwan that is negative for antibodies to HTLV-MA; (lane 5) serum from a representative *M. fascicularis* with malignant lymphoma; (lane 6) serum from healthy adult *M. cyclopis* from the NERPRC that is positive for antibodies to HTLV-MA; (lane 7) serum from a representative healthy adult *M. cyclopis* from the NERPRC that is negative for antibodies to HTLV-MA. The precipitates were eluted in a sample buffer containing 0.1*M* Cleland's reagent, 2 percent SDS, 0.08*M* tris-HCl, *p*H 6.8, 10 percent glycerol, and 0.2 percent bromophenol blue by boiling at 100°C for 2 minutes. Samples were analyzed in a 12.5 percent acrylamide resolving gel with 3.5 percent stacking gel according to the discontinuous buffer system of Laemmli (*23*). (B) Reactivity of serum samples with the glycoprotein preparation of Hut 102; the procedure was described in detail (*7*). The glycoproteins were prepared from a soluble cell lysate of Hut 102 made with RIPA buffer excluding sodium deoxycholate and by passing through a Lentil Lectin–Sepharose CL-4B (Pharmacia) column at the ratio of 20×10^6 cells to 1 to 2 ml of undiluted Lentil Lectin–Sepharose CL-4B. The glycoproteins were eluted from the column with a buffer consisting of 0.15*M* NaCl, 0.05*M* tris-HCl, *p*H 7.2, 1 percent Triton X-100, and 0.2*M* methyl-α-D-mannoside. The eluted bound fraction was reacted with 10 μl of test serum bound to Protein A beads. Samples of the same serum were used in the same lanes 1 to 7 as described in (A). The precipitates were eluted from Protein A beads and subjected to SDS-PAGE as described above.

ATLL in people represents a strong case for an etiological relation between a given virus and a human cancer (*5, 16*). The reported cases of lymphoid malignancies in macaques are not numerous (*17, 18*). However, there have been reports of multiple cases that occurred over brief intervals of time, suggesting that a transmissible agent could be involved (*14, 18, 19*).

The LPD observed in macaques have not been characterized according to the subpopulations of lymphocytes involved. Nevertheless, the seroepidemiological pattern we observed parallels that seen in people where the presence of antibodies to HTLV is associated with a greatly increased risk for the development of a particular form of lymphoma. Our current results indicate that an agent similar to HTLV is present in colonies of macaques where outbreaks of ML have been reported, and that more consideration should be given to the possible involvement of these agents in the causation of lymphoproliferative abnormalities in macaques.

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Nuclear Localization and DNA Binding Properties of a Protein Expressed by Human c-*myc* Oncogene

Abstract. *Antisera to the human cellular* myc *oncogene product were used to identify a human c-myc specific protein with a molecular weight of 65,000. Subcellular fractionation showed that the human c-myc protein is predominantly found in the cell nucleus. The p65K$^{c\text{-}myc}$ protein binds to double- and single-stranded DNA as measured by a DNA affinity chromatography assay.*

The oncogene v-*myc* of the avian myelocytomatosis viruses arose from a closely related gene (c-*myc*) found in all vertebrate animals (*1*). Altered expression of the c-*myc* gene is an important event in malignant cell transformation. Possible mechanisms whereby changes in c-*myc* gene expression have occurred include insertion of a strong retroviral promoter in the vicinity of c-*myc* (*2*), amplification of c-*myc* DNA (*3*), or chromosomal translocations which bring the c-*myc* gene in close vicinity to various immunoglobulin loci (*4*). The protein product of the v-*myc* oncogene has been studied in MC29 viral infected or transformed cell lines using an antiserum to the NH_2-terminal part of a polyprotein containing a v-myc protein at its COOH-terminal end. The MC29 transforming specific protein, p110K$^{gag\text{-}myc}$, with a molecular weight of 110,000 (110K) is a nuclear, phosphorylated protein that binds to double-stranded DNA (*5*). Subsequent studies with antisera specific for the protein product of the v-*myc* oncogene itself confirmed the results on the p110K$^{gag\text{-}myc}$ polyprotein and extended them to show nuclear localization for v-myc proteins unlinked to any structural viral antigen (*6*).

We used antisera to the human c-myc protein to identify a 65K protein expressed by the human c-*myc* gene (*7*). The p65K$^{c\text{-}myc}$ protein is expressed early in development and is evolutionarily conserved between different species. Increased levels of the p65K$^{c\text{-}myc}$ protein, as compared with normal cells, were observed in cells with amplified c-*myc* DNA or cells containing chromosomal translocations involving the c-*myc* gene (*8*). These results imply that the p65K$^{c\text{-}myc}$ protein plays an important role during c-*myc* oncogenesis. Furthermore, we showed that the p65K$^{c\text{-}myc}$ protein is an inducible gene product in the sense that synthesis of this protein is greatly enhanced after activation of cells with mitogens. We now report that the human p65K$^{c\text{-}myc}$ protein has two properties in common with the MC29 viral transforming polyprotein, believed to be relevant for viral transformation.

Three different antisera to human c-myc protein were used for the identification of the p65K$^{c\text{-}myc}$ protein (*7*). One of these anti(c-myc N-ter) was a rabbit antiserum to a synthetic peptide representing amino acids 24 to 40 of the human c-myc protein. The other two were raised against c-*myc* antigens expressed as fusion proteins in *Escherichia coli* containing either amino acids 42 to 180 anti(c-myc 5′ exon) or amino acids 380 to 439 anti(c-myc C-ter) of the human c-myc protein. Two of these antisera readily immunoprecipitated the p65K$^{c\text{-}myc}$ protein whereas the other, anti(c-myc 5′ exon), only weakly immunoprecipitated this protein. In addition, anti(c-myc 5′ exon) immunoprecipitated a 49K pro-