JOURNAL OF CLINICAL MICROBIOLOGY, June 1995, p. 1510–1515 Vol. 33, No. 6
0095-1137/95/$04.00+0

Inhibitory Activity in Saliva of Cell-to-Cell Transmission of Human T-Cell Lymphotropic Virus Type 1 In Vitro: Evaluation of Saliva as an Alternative Source of Transmission

TARO YAMAMOTO,[1] KAORU TERADA,[1] NORIYUKI NISHIDA,[1] RYOZO MORIUCHI,[1] SUSUMU SHIRABE,[2] TATSUFUMI NAKAMURA,[2] YOSHIRO TSUJI,[3] TSUTOMU MIYAMOTO,[1] AND SHIGERU KATAMINE[1]*

Department of Bacteriology,[1] First Department of Internal Medicine,[2] and Department of Pediatrics,[3] Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan

Received 6 December 1994/Returned for modification 2 February 1995/Accepted 9 March 1995

Human T-cell lymphotropic virus type 1 (HTLV-1) is known to be transmitted vertically through breast-feeding and horizontally by blood transfusion and sexual contact. Our intervention study has suggested the presence of additional alternative maternal transmission pathways. To explore the possibility of transmission through saliva, we used PCR to quantify the HTLV-1 provirus in saliva samples from 18 carrier mothers and 10 patients with HTLV-1-associated myelopathy/tropical spastic paraparesis. The provirus was detected in 60 and 90%, respectively, of the samples, with estimated copy numbers in the range of 10 to 10^4/ml. However, the saliva, regardless of the presence or absence of antibodies to the virus, showed a strong tendency to inhibit the cell-to-cell transmission of HTLV-1 in vitro, as examined by a syncytium inhibition assay. The natural inhibitory activity in saliva of seronegative volunteers was heat sensitive, and most of the activity was recovered by ultrafiltration in the fraction of macromolecules with a molecular weight of more than 100,000. In addition to this natural activity, saliva of HTLV-1-infected individuals contained immunoglobulin G molecules capable of neutralizing syncytium formation. These results strongly suggested that HTLV-1-infected cells in the carriers' saliva, which contains neutralizing antibodies in addition to the natural activity inhibiting cell-to-cell viral infection, barely transmit the virus. Transmission of HTLV-1 through the saliva would thus seem to be rare, if it occurs at all.

Human T-cell lymphotropic virus type 1 (HTLV-1) is a causative agent of adult T-cell leukemia (12, 25) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (7, 23). The virus is transmitted through both horizontal and vertical pathways (1). The major routes of horizontal transmission are thought to be through blood transfusion (8, 22) and sexual contact from male to female (28). Sexual transmission via seminal fluid is evidenced by epidemiological data showing that most of the wives of carrier husbands were infected but not vice versa in a Japanese cohort (28) and by the presence of HTLV-1-infected cells in carriers' seminal fluid (21). However, one-way male-to-female transmission is not always the case in other populations (19). It is reasonable to consider that unidentified pathways are involved in horizontal transmission of the virus. Vertical transmission of HTLV-1 occurs mostly through postnatal breast-feeding (10, 30). In an attempt to break the cycle of mother-to-child transmission, in 1987 we started an intervention program in Nagasaki Prefecture, which is located in the westernmost part of mainland Japan where HTLV-1 is highly endemic (9, 11). The program involves screening pregnant women for HTLV-1 carriers, requiring that refrain carrier mothers from breast-feeding, and follow-up the children of carrier mothers. The incidence of maternal transmission, which had been 20 to 30% before the program was started, has dramatically declined. However, approximately 3% of carrier mothers' children appeared to be infected despite formula feeding (9, 15). This indicated the presence of alternative mother-to-child transmission pathways, among which intrauterine, perinatal, and postnatal pathways are all possible candidates. However, we recently demonstrated that none of the cord blood samples from nine babies who were born to carrier mothers and were formula fed and later confirmed to be infected with HTLV-1 were positive for the proviral DNA sequence as examined by a highly sensitive PCR (14, 15). This would argue against intrauterine transmission of HTLV-1.

The present study has focused on saliva as a source of HTLV-1 transmission. Because HTLV-1 is transmitted only in a cell-to-cell manner (22), the source would need to contain a significant number of infected cells. Saliva contains various types of cellular components, including lymphocytes. Miyoshi et al. have demonstrated the presence of proviral DNA in the saliva of HTLV-1-infected individuals (18). Saliva could be transmitted from person to person by kissing or sharing tableware. Additionally, it is a Japanese custom for mothers to give food chewed by themselves to their children during the weaning period. Saliva could thus be considered one of the most likely candidates as a source of infection other than blood, seminal fluid, and breast milk. Here, we have evaluated this possibility by analyzing the viral load and titer of anti-HTLV-1 antibodies and their neutralizing activities in saliva samples from infected individuals, as well as the effect of normal whole saliva on the cell-to-cell transmission of the virus in vitro.

MATERIALS AND METHODS

Subjects. Saliva and serum samples were obtained from 10 HAM/TSP patients (one male and nine females; age [mean ± standard error], 60 ± 8.1 years), 18 carrier mothers (age, 31.5 ± 0.9 years), and two male seronegative volunteers (age, 26.5 ± 1.5 years). Seropositivity was screened by using a particle agglutination kit (Serodia HTLV-1; Fuji-Rebio, Tokyo, Japan) (5) and confirmed by immunoblotting, using the MT-2 cell lysate as an antigen (17). Diagnosis of HAM/TSP was made after the strict criteria of Nagasaki University Hospital were met. No individual had overt salivary dysfunction. Informed consent was

* Corresponding author. Phone: 81-958-49-7059. Fax: 81-958-49-7060.

obtained from every individual examined, and the study was conducted in accordance with the human experimentation guidelines of the authors' institution.

Saliva. Unstimulated whole saliva was collected in a plastic centrifuge tube and placed on ice. The collected saliva was usually centrifuged (2,000 × *g*) for 15 min at 4°C. The resulting supernatant was aliquoted and stored at −80°C. Before use, the saliva sample was thawed and passed through a filter (pore size, 0.45 μm). Uncentrifuged fresh whole saliva was used for the purpose of DNA extraction.

Titration of serum and salivary antibodies to HTLV-1. Titers of antibodies to HTLV-1 in serum and saliva samples were determined by testing twofold serial dilutions of the samples with the particle agglutination kit (Serodia HTLV-1).

Semiquantitative determination of HTLV-1 viral load in saliva. DNA was isolated from 1 ml of the uncentrifuged fresh saliva by adding 0.2 ml of sixfold-concentrated DNA extraction buffer to give final concentrations of 10 mM Tris-HCl (pH 8.2), 2 mM EDTA, 150 mM NaCl, 0.5% sodium dodecyl sulfate (SDS), and 0.4 mg of proteinase K per ml. The mixture was incubated at 37°C for 16 h, and DNA was extracted by treatment with phenol-chloroform. Amounts of DNA isolated from 1 ml of saliva varied among samples and ranged between 10 and 100 μg. The DNA concentration was adjusted in TE buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA) at 100 μg/ml and was serially diluted twofold. Various amounts (range, 0.1 to 0.2 μg) of DNA were subjected to two-step PCR with nested primer pairs targeting the pX region of the HTLV-1 genome, as described previously (29), and the products were detected in an agarose gel stained with ethidium bromide. Since the nested PCR could detect a single molecule of HTLV-1 proviral DNA (29), the copy number in 1 μg of DNA was estimated from the endpoint that amplified the DNA. Subsequently, the viral load in saliva (copies per milliliter) was calculated on the basis of the amount of DNA isolated from each saliva sample.

Ultrafiltration of saliva. Ultrafiltration was performed with commercial microconcentrators, Centricon 30 and 100 (Amicon, Danvers, Mass.), with membranes with 30,000- and 100,000-molecular-weight cutoffs, respectively. A filtered saliva sample (1 ml) was applied to the microconcentrator and centrifuged (3,000 × *g*) for 30 min at 4°C. The resulting retentate and filtrate were recovered, adjusted to the original volume with phosphate-buffered saline (PBS), and subjected to the assay.

Purification of salivary IgG. Immunoglobulin G (IgG) of pooled saliva samples from HAM/TSP patients and seronegative volunteers was independently purified by ammonium sulfate precipitation coupled with protein G-affinity column chromatography. The saliva was centrifuged (20,000 × *g*) for 30 min at 4°C. Saturated ammonium sulfate solution was added to the supernatant to a final concentration of 45% (vol/vol). The resulting precipitate was recovered by centrifugation (20,000 × *g*) for 1 h at 4°C, dissolved in PBS, and dialyzed against PBS. The dialyzed solution was subsequently applied to a commercial column of protein G-coupled Sepharose beads (Immuno-Pure [G]; Pierce, Rockford, Ill.). IgG bound to the Sepharose beads was eluted according to a protocol provided by the manufacturer. The purified IgG was quantified with a protein assay kit (Bio-Rad, Richmond, Calif.). The quality and quantity of the IgG were finally confirmed in an SDS-polyacrylamide gel stained with Coomassie brilliant blue.

Syncytium inhibition assay. The cell lines used in the assay were XC (16), a rat sarcoma cell line with Rous sarcoma virus, and an HTLV-1-producing cell lines, C91/PL (26). XC cells were cultured in a 48-well flat-bottom plate for 24 h at a concentration of 4×10^4 cells per 0.5 ml of RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum. The medium was then removed, and 4×10^4 C91/PL cells were added to each well with or without various concentrations of whole saliva or salivary IgG to a final volume of 0.5 ml of the medium containing 4 μg of Polybrene per ml. After cultivation for 16 h, cells were fixed with methanol and stained with Giemsa. All syncytia formed in the wells were counted under a phase-inverted microscope. Cells with five or more nuclei were scored as syncytia, as previously described (20). Each experiment was carried out in triplicate. The level of inhibition at each concentration of the sample (percent inhibition) was calculated from the decrease in the number of syncytia compared with cultures without inhibitors, which usually gave 200 to 300 syncytia. Finally, the inhibitory activity of the sample was expressed as the concentration allowing 50% inhibition of the number of syncytia (IC_{50}) estimated on the basis of an inhibition curve.

RESULTS

Detection of HTLV-1 proviral DNA in saliva samples from carrier mothers. The HTLV-1 proviral DNA in saliva samples from 18 carrier mothers and 10 HAM/TSP patients was examined semiquantitatively by PCR targeting the pX region of the viral genome. HTLV-1 was detectable in 11 (60%) and 9 (90%) samples from carrier mothers and HAM/TSP patients, respectively. Copy numbers ranged between 10 and 10^4/ml, and geometric mean values in the two groups were 1.7 (95% confidence interval, 1.5 to 2.1) and 2.5 (95% confidence interval, 2.1 to 3.0) $\log_{10}$ copies per ml, respectively (Fig. 1).

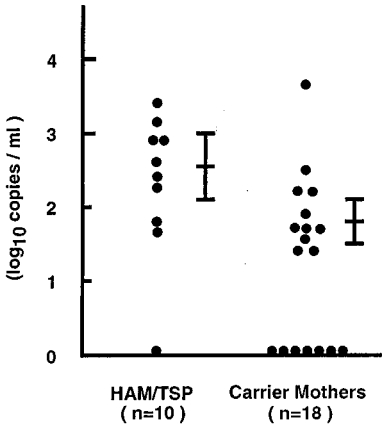

FIG. 1. HTLV-1 viral loads in saliva samples from HAM/TSP patients and carrier mothers. The copy number of the HTLV-1 provirus in 1 ml of each sample was determined by PCR as described in the text. Geometric values ($\log_{10}$ copies per milliliter) are plotted. Vertical bars indicate means and 95% confidence intervals of PCR-positive samples from nine HAM/TSP patients and 11 carrier mothers.

Detection of anti-HTLV-1 antibodies in saliva. The saliva of infected individuals contains antibodies to HTLV-1. Our previous study indicated that the major class of anti-HTLV-1 antibodies in the saliva was IgG (29). These antibodies are assumed to have the potential to neutralize HTLV-1 infectivity. By the particle agglutination test, antibodies to HTLV-1 were detectable in saliva samples from 14 of 18 carrier mothers and 8 of 10 HAM/TSP patients. The antibodies in the saliva were titrated by testing serial twofold dilutions, and the titers were compared with those of the sera of the same individuals (Fig. 2). Antibody titers of the saliva were lower than those of the sera, but significant correlation was observed between the two (Pearson's product moment correlation coefficient, $r^2 = 0.61$; $P < 0.001$).

Neutralizing effect of salivary IgG of infected individuals on cell-to-cell transmission of HTLV-1 in vitro. We compared the syncytium inhibitory activities of saliva samples from two se-

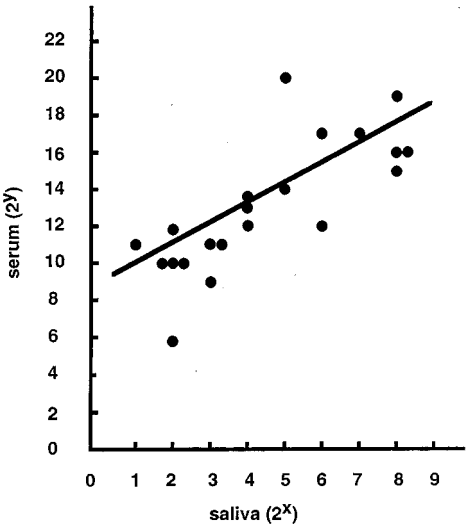

FIG. 2. Correlation of titers of serum and salivary antibodies to HTLV-1. The antibodies in serum and saliva samples from 21 infected individuals were titrated by the particle agglutination test. Correlation was significant by Pearson's product moment correlation coefficient ($r^2 = 0.61$; $P < 0.001$).

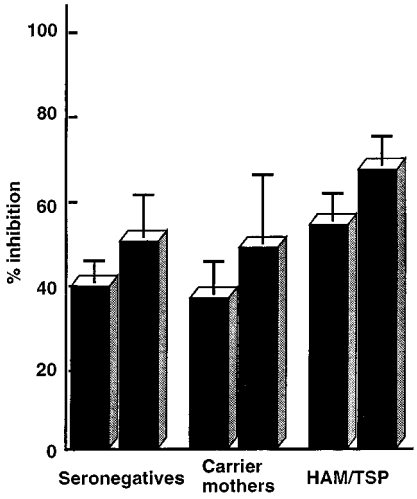

FIG. 3. Comparison of the syncytium inhibitory activities among saliva samples from seronegative volunteers, carrier mothers, and HAM/TSP patients. Samples from two individuals of each group were diluted 64-fold, and their inhibitory activities (percent inhibition) were determined by comparison with saliva-less cultures by the syncytium inhibition assay. Experiments were performed in triplicate, and the results are shown as means ± standard errors.

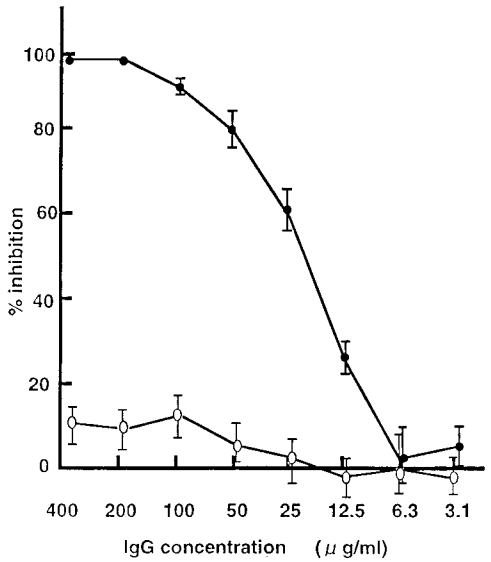

FIG. 4. Neutralizing potential of salivary IgG of HAM/TSP patients. Various concentrations of IgG purified from pooled whole saliva of HAM/TSP patients (closed circles) and seronegative volunteers (open circles) were examined in the syncytium inhibition assay. Experiments were performed in triplicate, and the results are shown as means ± standard errors.

ronegative volunteers, two carrier mothers (salivary anti-HTLV-1 titers, 2^2 each), and two HAM/TSP patients (salivary anti-HTLV-1 titers, 2^8 each). Whole saliva and HTLV-1-producing C91/PL cells were simultaneously added to target XC cells, and the number of syncytia formed was counted after incubation for 16 h. All undiluted saliva samples, including those from seronegative volunteers, completely inhibited syncytium formation. The samples were then diluted 2^6, and their inhibitory activities were determined by comparison with those of the saliva-less culture. As shown in Fig. 3, the saliva samples from the HAM/TSP patients showed higher activity (53.4% ± 3.6% and 66.5% ± 3.8%), suggesting a possible neutralizing activity of salivary antibodies to HTLV-1. However, the saliva samples from the seronegative volunteers also significantly inhibited syncytium formation, with inhibition levels (39.7% ± 2.9% and 49.8% ± 5.9%) that were similar to those of the samples from the two carrier mothers (38.6% ± 4.5% and 47.8% ± 9.5%). These results strongly suggested the presence of a natural factor, other than antibodies to HTLV-1, capable of inhibiting HTLV-1 infectivity in saliva. All saliva samples from five other independent seronegative volunteers revealed similar inhibitory activity (data not shown).

To further evaluate the neutralizing activity of salivary antibodies to HTLV-1, the natural inhibitory activity was eliminated from pooled saliva from both HAM/TSP patients and normal volunteers by ammonium sulfate precipitation and subsequent protein G-affinity column chromatography, and the neutralizing activities of the purified IgG were directly examined. As shown in Fig. 4, the salivary IgG of healthy volunteers did not show any activity even at a concentration of 0.4 mg/ml, which indicated that the inhibitor present in the normal saliva was not an IgG molecule. In contrast, that of HAM/TSP patients revealed potent neutralizing activity, with an IC_{50} of 0.02 mg/ml.

Inhibitory effect of normal saliva on HTLV-1-induced syncytium formation. To characterize the natural inhibitory activity in the saliva, serially twofold-diluted whole saliva from a seronegative volunteer was subjected to the syncytium inhibition assay. Saliva at dilutions of 2^{-1} to 2^{-5} completely inhibited syncytium formation, and a significant inhibition was observed at dilutions up to 2^{-7} (Fig. 5a). The IC_{50} was estimated to be $2^{-5.5}$. This inhibition was unlikely to be due to a cytotoxic or cytostatic effect, because the saliva, even at a dilution of 2^{-1}, was not toxic to C91/PL cells (Fig. 5b) and did not affect cell growth (data not shown) during incubation. The saliva was then subjected to various physical treatments whose effects were determined by the syncytium inhibition assay (Table 1). A single freezing-and-thawing showed a slight effect, but additional cycles (five cycles) resulted in a 34% loss of the activity. A more profound effect was brought about by heat treatment at 56°C for 30 min, which reduced the activity by 16%. To roughly estimate the molecular weight of the components exerting the inhibitory activity, the saliva was subjected to ultrafiltration. At a molecular weight cutoff of 100,000, the activity was retained in the retentate, with the filtrate containing activity of less than 6%. Furthermore, the retentate revealed more potent activity than the original whole saliva (Table 1).

DISCUSSION

HTLV-1 proviral DNA was detected in 60 and 90%, respectively, of saliva samples from carrier mothers and HAM/TSP patients. Previously, Miyoshi et al. reported the detection by PCR of HTLV-1 in all saliva specimens from infected individuals (18). The lower frequency of detection in the present study might be due to the method used to isolate salivary DNA. While the other group used salivary cells, we isolated DNA from whole saliva for the purpose of quantification of the proviral DNA on the basis of saliva volume. The yield of DNA from 1 ml of whole saliva varied between 10 and 100 μg. Since we subjected a maximum of 1 μg of DNA to PCR, we actually analyzed DNA equivalent to 0.01 to 0.1 ml of saliva. It is therefore likely that small amounts of HTLV-1 present in a significant part of the PCR-negative samples escaped our detection. Isolation of DNA from the immune cell population of saliva samples might allow the demonstration of HTLV-1 in a higher percentage of the individuals studied. The presence of

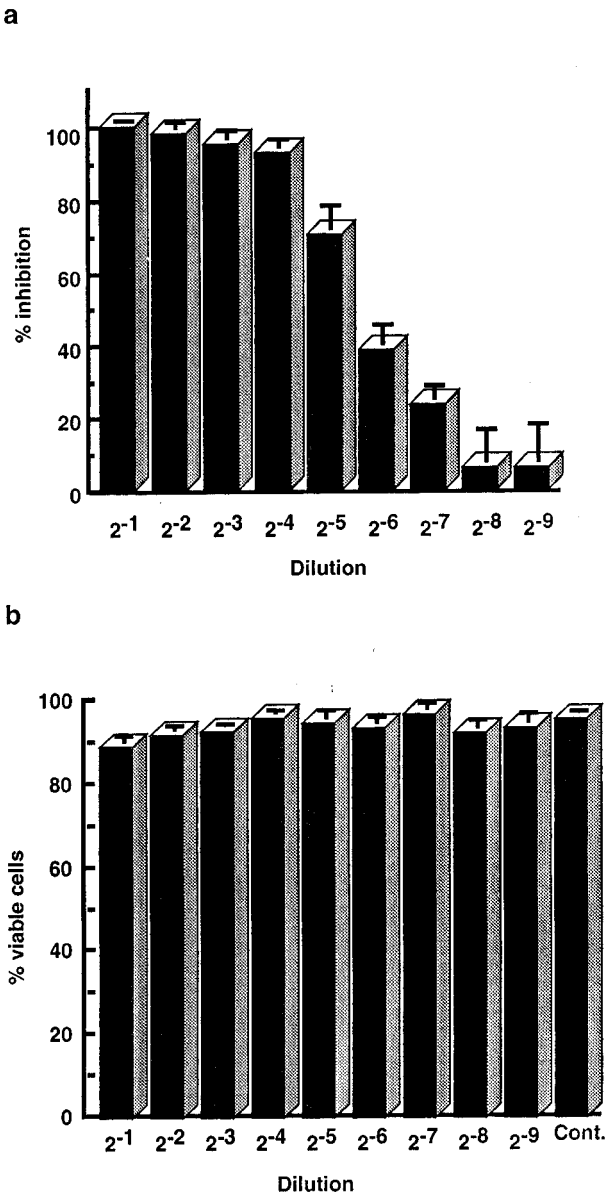

FIG. 5. Natural inhibitory activity in saliva of cell-to-cell transmission of HTLV-1. (a) Serial twofold dilutions of filtered whole saliva of a seronegative volunteer were mixed with HTLV-1-producing C91/PL cells, and the mixture was added to a culture of XC cells. Syncytia in the culture were counted after 16 h. The inhibitory activity (percent inhibition) was calculated by comparison with the culture without saliva. (b) Cytotoxicity to C91/PL of the diluted saliva was determined by a dye exclusion test. All experiments were performed in triplicate, and the results are shown as means ± standard errors.

HTLV-1 in most of the saliva samples from infected individuals would appear to support the hypothesis that saliva is an alternative source of HTLV-1 transmission. However, mean values of the viral load in the PCR-positive saliva of carriers and HAM/TSP patients were not high, at 1.7 and 2.5 $\log_{10}$ copies per ml, respectively. These levels are at least 2 orders of magnitude lower than those in peripheral blood of the same carrier and disease populations that were previously estimated (29). Breast milk is a well-known source of transmission of HTLV-1. We previously estimated the concentration of HTLV-1-bearing cells in the milk to be on the order of 10^3 cells per ml on the basis of the number of viral protein-expressing

TABLE 1. Effects of various treatments on HTLV-1 inhibitory activity of saliva

Treatment	IC_{50} ($\log_2$)[a]	Relative inhibitory activity (%)
None	5.5	100
Freezing and thawing[b]		
One cycle	5.2	81
Five cycles	4.9	66
Heating, 56°C, 30 min	2.9	16
Centricon 30 (mol wt)[c]		
Filtrate (<30,000)	1.6	6
Retentate (>30,000)	6.0<	141<
Centricon 100 (mol wt)[c]		
Filtrate (<100,000)	1.2	5
Retentate (>100,000)	6.0<	141<

[a] Estimated from an inhibition curve obtained by syncytium inhibition assay.
[b] Freezing at −20°C and thawing at room temperature.
[c] The filtrate and retentate were adjusted to the original volume before assay.

cells present after short-term culture (30). This could be an overestimation, as the infected cell population is known to sometimes expand in the culture. However, it is conceivable that breast milk and saliva of an infected individual contain roughly equivalent concentrations of infected cells. Since a baby is estimated to be fed about 500 ml of milk per day for around 200 days, a very large number of HTLV-1-infected cells could be transferred orally from the mother (10). In contrast, the volume of saliva and the number of infected salivary cells potentially transferred from mothers to children or between sexual partners are presumed to be much smaller.

Normal saliva was shown to inhibit the cell-to-cell transmission of HTLV-1 in vitro in a dose-dependent manner, with an IC_{50} of $2^{-5.5}$. This activity was not due to salivary particulate material such as cells, bacteria, food debris, and aggregates of salivary proteins, since the saliva samples were subjected to the assay after centrifugation at 2,000 × *g* for 15 min and filtration through a 0.45-μm-pore-size filter to remove the particulates. The activity was heat (56°C) sensitive, suggesting that some bioactive components are involved. However, these are unlikely to be cytotoxic or cytostatic factors, since saliva even at a high concentration showed neither a toxic nor a static effect on the cultured cells under the experimental conditions. Increased cycles of freezing and thawing partially abrogated the activity. This suggests that the activity is associated with a macromolecule whose structure can be altered by extensive freezing and thawing. Ultrafiltration of the saliva gave a result consistent with this notion. The inhibitory activity was retained in the fraction of macromolecules with a molecular weight of more than 100,000. Interestingly, this fraction revealed a relative activity clearly higher than that of the original saliva. This may indicate the presence of a low-molecular-weight component which regulates the syncytium inhibitory activity in the saliva. Previously, the inhibition of human immunodeficiency virus type 1 (HIV-1) infection was demonstrated in whole human and chimpanzee saliva (3, 4, 16). However, this activity is unlikely to be the same one that inhibits the HTLV-1-induced syncytium formation in vitro, because the anti-HIV activity was recovered for the most part in the particulate fraction and was resistant to heating at 56°C (4). Mechanisms of syncytium inhibition by saliva remain to be elucidated. Attachment of HTLV-1 envelope glycoprotein to the surface of target cells is thought to be primarily responsible for syncytium formation (2, 24). The saliva may directly inactivate the virus on the cell surface of infected cells or inhibit the interaction between the viral envelope protein and unidentified putative receptor or

coreceptor molecules. Alternatively, the saliva may act on certain cellular molecules involved in the subsequent complex process of cell fusion. Identification of the salivary molecule responsible for the syncytium inhibitory activity will provide new insight into the understanding of molecular mechanisms in the early phase of HTLV-1 infection. Potential candidate molecules are found among the complement proteins, which are easily inactivated by heat treatment. Some animal retroviruses have been shown to activate the human classical complement pathway by an antibody-independent mechanism which results in the lysis of virions. Previously, antibody-independent binding of complement component C1q to HTLV-1 was also demonstrated (27). The role of salivary complement proteins in syncytium inhibition should be evaluated.

Anti-HTLV-1 antibodies were frequently detectable in the saliva of carrier mothers and HAM/TSP patients, and titers of these antibodies showed a significant correlation with the titers of serum antibodies. Our previous study suggested that the major class of these antibodies in the saliva was IgG (29). The present study confirmed that salivary IgG of infected individuals had the potential to neutralize the cell-to-cell transmission of HTLV-1. Purified salivary IgG of HAM/TSP patients inhibited syncytium formation in a dose-dependent manner, with an IC_{50} of 0.02 mg/ml. This concentration is much lower than the natural IgG concentration in whole saliva, which ranged between 0.5 and 30 mg/ml (13). It is therefore likely that salivary anti-HTLV-1 antibodies contribute to the prevention of cell-to-cell transmission of the virus in the oral cavity.

Our results argue against the transmission of HTLV-1 through saliva, although the possibility cannot be ruled out completely. HTLV-1-infected cells in carriers' saliva, which contains neutralizing antibodies in addition to the natural activity inhibiting cell-to-cell viral infection, barely transmit the virus. Moreover, the number of infected cells potentially transferred through the saliva is much smaller than the number transferred through breast milk. Oral transmission of HTLV-1 other than through breast milk may thus be presumed to be very rare. Well-controlled epidemiological studies are needed to confirm our conclusion.

Alternative pathways of HTLV-1 transmission remain to be elucidated. Female-to-male horizontal transmission is more frequent in countries where other sexually transmissible diseases, including HIV infection, are common. These may cause an increase in vulnerability to HTLV-1. Among vertical pathways, the perinatal transmission of HTLV-1 has yet to be evaluated, although that of HIV and hepatitis B virus is well established. These two viruses transmit in a cell-free manner, in contrast to the cell-to-cell infection of HTLV-1. Even so, the perinatal pathway should be evaluated as a likely candidate for alternative HTLV-1 transmission.

ACKNOWLEDGMENTS

We thank Shigeo Hino for critical discussion and Kyoko Kubota and Kenji Kurokawa for technical assistance. We are also grateful to Amanda Nishida for help in manuscript preparation.

Part of this work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. **Blattner, W. A.** 1990. Epidemiology of HTLV-1 and associated diseases, p. 251–264. *In* W. A. Blattner (ed.), Human retrovirology HTLV. Raven Press, Ltd., New York.
2. **Deramarre, L., C. Pique, D. Pham, T. Turtz, and M. C. Dokhelar.** 1994. Identification of functional regions in the human T-cell leukemia virus type 1 SU glycoprotein. J. Virol. **68:**3544–3549.
3. **Fox, P. C., A. Wolff, C.-K. Yeh, J. C. Atokinson, and B. J. Baum.** 1988. Saliva inhibits HIV-1 infectivity. J. Am. Dent. Assoc. **116:**635–637.
4. **Fox, P. C., A. Wolff, C.-K. Yeh, J. C. Atokinson, and B. J. Baum.** 1989. Salivary inhibition of HIV-1 infectivity: functional properties and distribution in men, women, and children. J. Am. Dent. Assoc. **118:**709–711.
5. **Fujino, R., K. Kawato, M. Ikeda, M. Miyakoshi, and J. Imai.** 1991. Improvement of gelatine particle agglutination test for detection of anti-HTLV-1 antibody. Jpn. J. Cancer Res. **82:**367–370.
6. **Fultz, P. N.** 1986. Components of saliva inactivate human immunodeficiency virus. Lancet **ii:**1215.
7. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, and A. Calender.** 1985. Antibodies to human T-lymphotropic virus type I in patients with tropical spastic paraparesis. Lancet **ii:**407–410.
8. **Hino, S., T. Kawamichi, M. Funakoshi, M. Kanamura, T. Kitamura, and T. Miyamoto.** 1984. Transfusion-mediated spread of the human T-cell leukemia virus in chronic hemodialysis patients in a heavily endemic area, Nagasaki. Gann **75:**1070–1075.
9. **Hino, S., H. Sugiyama, H. Doi, T. Ishimaru, T. Yamabe, T. Tsuji, and T. Miyamoto.** 1987. Breaking the cycle of HTLV-1 transmission via carrier mothers' milk. Lancet **ii:**158–159.
10. **Hino, S., K. Yamaguchi, S. Katamine, H. Sugiyama, T. Amagasaki, K. Kinoshita, Y. Yoshida, H. Doi, Y. Tsuji, and T. Miyamoto.** 1985. Mother-to-child transmission of human T-cell leukemia virus type I. Jpn. J. Cancer Res. **76:**474–480.
11. **Hinuma, Y., H. Komoda, T. Chosa, T. Kondo, M. Kohakura, T. Takenaka, M. Kikuchi, M. Ichimaru, K. Yuniki, I. Sato, R. Matsuo, Y. Takiuchi, H. Uchino, and M. Hanaoka.** 1982. Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nationwide seroepidemiologic study. Int. J. Cancer. **29:**631–635.
12. **Hinuma, Y., K. Nagata, M. Hanaoka, T. Nakai, K. Matsumoto, S. Kinoshita, S. Shirakawa, and I. Miyoshi.** 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.
13. **Ichikawa, S., and A. Itoh.** 1993. Detection of antibodies to HIV-genes in saliva and urine. Nippon Rinshou **51:**299–305.
14. **Katamine, S., R. Moriuchi, T. Yamamoto, K. Terada, K. Eguchi, Y. Tsuji, T. Yamabe, T. Miyamoto, and S. Hino.** 1994. Significance of HTLV-1 proviral DNA in umbilical cord blood of babies born to carrier mothers. Lancet **343:**1326–1327.
15. **Kawase, K., S. Katamine, R. Moriuchi, T. Miyamoto, K. Kubota, H. Igarashi, H. Doi, Y. Tsuji, T. Yamabe, and S. Hino.** 1992. Maternal transmission of HTLV-1 other than through breast milk: discrepancy between the polymerase chain reaction positivity of cord blood samples for HTLV-1 and subsequent seropositivity of individuals. Jpn. J. Cancer Res. **83:**968–977.
16. **Klement, V., W. P. Rowe, J. W. Hartley, and W. E. Pugh.** 1969. Mixed culture cytopathogenicity: a new test for growth of murine leukemia viruses in tissue culture. Proc. Natl. Acad. Sci. USA **63:**753–758.
17. **Miyoshi, I., M. Kubonishi, S. Yoshimoto, T. Akagi, Y. Otuki, Y. Shiraishi, and Y. Hinuma.** 1981. Type C virus particles in a cord blood T cell line derived by cocultivating normal human cord leukocytes and human leukemic T cells. Nature (London) **294:**770–771.
18. **Miyoshi, I., T. Sawada, Y. Iwahara, K. Ishii, I. Kubonishi, and H. Taguchi.** 1992. Excretion of HTLV-1 in saliva. JAMA **267:**236.
19. **Murphy, E. L., J. P. Figueroa, W. L. Gibbs, A. Brathwaite, M. Holding-Cobham, D. Waters, B. Hanchard, and W. A. Blattner.** 1989. Sexual transmission of human T-lymphotropic virus type-1. Ann. Intern. Med. **111:**555–560.
20. **Nagy, K., P. Clapham, C.-P. Rachanee, and R. A. Weiss.** 1983. Human T-cell leukemia virus type 1: induction of syncytia and inhibition by patients' sera. Int. J. Cancer **32:**321–328.
21. **Nakano, S., Y. Ando, M. Ichijo, I. Moriyama, S. Saito, K. Sugamura, and Y. Hinuma.** 1984. Search for possible routes of vertical and horizontal transmission of adult T-cell leukemia virus. Gann **75:**1044–1045.
22. **Okochi, K., H. Sato, and Y. Hinuma.** 1985. A retrospective study on transmission of adult T cell leukemia virus by blood transfusion: sero-conversion in recipients. Vox Sang **46:**245–253.
23. **Osame, M., K. Usuku, S. Izumo, N. Ijichi, H. Aminitani, A. Igata, M. Matsumoto, and M. Tara.** 1986. HTLV-1 associated myelopathy, a new clinical entity. Lancet **i:**1031–1032.
24. **Pique, C., D. Pham, T. Tursz, and M. C. Dokhelar.** 1992. Human T-cell leukemia virus type 1 envelope protein maturation process: requirements for syncytium formation. J. Virol. **66:**906–913.
25. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1981. 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.
26. **Popovic, M., P. S. Sarine, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, and R. C. Gallo.** 1983. Isolation and transmission of human retrovirus (human T cell leukemia virus). Science **219:**856–859.
27. **Spear, T. S., H. Jiang, B. L. Sullivan, H. Gewurz, A. L. Landay, and T. F. Lint.** 1991. Direct binding of complement component C1q to human immunodeficiency virus and human T lymphotropic virus-I coinfected cells. AIDS Res. Hum. Retroviruses **7:**579–585.
28. **Tajima, K., S. Tominaga, T. Suchi, T. Kawagoe, H. Komoda, Y. Hinuma, T.**

Oda, and K. Fujita. 1982. Epidemiological analysis of the distribution of antibody to adult T-cell leukemia-virus-associated antigen: possible horizontal transmission of adult T-cell leukemia virus. Gann **73:**893–901.

29. **Terada, K., S. Katamine, K. Eguchi, R. Moriuchi, M. Kita, H. Shimada, I. Yamashita, K. Iwata, Y. Tsuji, S. Nagataki, and T. Miyamoto.** 1994. Prevalence of serum and salivary antibodies to HTLV-1 in Sjogren's syndrome. Lancet **344:**1116–1119.
30. **Yamanouchi, K., K. Kinoshita, R. Moriuchi, S. Katamine, T. Amagasaki, S. Ikeda, M. Ichimaru, T. Miyamoto, and S. Hino.** 1985. Oral transmission of human T-cell leukemia virus type-1 into a common marmoset (Callithrix Jacchus) as an experimental model for milk-borne transmission. Jpn. J. Cancer Res. **76:**481–487.