A novel mother-to-child human T-cell leukaemia virus type 1 (HTLV-1) transmission model for investigating the role of maternal anti-HTLV-1 antibodies using orally infected mother rats

Yuji Murakami,1 Atsuhiko Hasegawa,1,* Satomi Ando,1 Reiko Tanaka,2 Takao Masuda,1 Yuetsu Tanaka2 and Mari Kannagi1

Abstract

Human T-cell leukaemia virus type 1 (HTLV-1) is a human retrovirus that is a causative agent of adult T-cell leukaemia/lymphoma (ATL) and is mainly transmitted from an infected mother to her child via breastfeeding. Such an HTLV-1 infection during childhood is believed to be a risk factor for ATL development. Although it has been suggested that an increased proviral load (PVL), a higher titre of antibody (Ab) in the infected mother and prolonged breastfeeding are associated with an increased risk of mother-to-child transmission (MTCT), the mechanisms underlying MTCT of HTLV-1 remain largely unknown. In this study, we developed an MTCT model using orally HTLV-1-infected rats that have no Ab responses against viral antigens, such as Gag and Env. In this model, HTLV-1 could be transmitted from the infected mother rats to their offspring at a high rate (50–100 %), and the rate of MTCT tended to be correlated with the PVL of the infected mother rats. Furthermore, passive immunization of uninfected adult rats and an infected mother rat with a rat anti-HTLV-1 Env gp46-neutralizing mAb was unable to suppress primary oral HTLV-1 infection to the adult rats and vertical HTLV-1 transmission to the offspring, respectively. Our findings indicate that this MTCT model would be useful to investigate not only the mechanisms of MTCT but also the role of anti-HTLV-1 Ab in MTCT of HTLV-1. They also provide some information on the role of maternal Abs in MTCT, which should be considered when designing a strategy for prevention of MTCT of HTLV-1.

INTRODUCTION

Human T-cell leukaemia virus type 1 (HTLV-1) is a causative agent of a highly aggressive CD4+ T-cell malignancy known as adult T-cell leukaemia/lymphoma (ATL) and an inflammatory neurological disease designated as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1–3]. Approximately 10–20 million individuals have been infected with HTLV-1 worldwide, mainly in highly endemic areas such as south-western Japan, the Caribbean, South America, Melanesia and equatorial Africa [4]. Among infected individuals, approximately 5 % of them will develop ATL after a long latent period of 40–50 years [5].

HTLV-1 can be vertically or horizontally transmitted via cell-to-cell contact with contaminated body fluids, such as breast milk, semen and blood. Numerous studies have demonstrated that mother-to-child transmission (MTCT) through breastfeeding is the predominant route of HTLV-1 infection [6–9]. Such an HTLV-1 infection early in life has been thought to be a potential risk factor for the development of ATL [9]. In addition, a previous study by us demonstrated that adult rats orally infected with HTLV-1 carried a higher proviral load (PVL), which is one of the risk factors for ATL development [10], when compared to rats with intraperitoneal infection [11]. Therefore, it is very important to fully understand the mechanism of MTCT through breastfeeding for preventing HTLV-1 infection during childhood.

In MTCT, prolonged breastfeeding is known to raise the probability of HTLV-1 infection among children [12]. Several epidemiological studies have indicated that a higher
PVL in PBMCs and breast milk is a good predictor of the increased risk of MTCT [13, 14]. This evidence indicates that the risk of MTCT will be increased by multiple exposures over time, with many infected cells at the mucosal tissues of the digestive tract. In addition to PVL, an elevated titre of anti-HTLV-1 antibody (Ab) in infected mothers has been suggested to be involved in an increased risk of MTCT [15]. However, although the titre of anti-HTLV-1 Abs may be correlated with PVL in most HTLV-1-infected individuals [14], the role of anti-HTLV-1 Ab in MTCT is not fully understood.

HTLV-1 MTCT has also been reported in some animal models, as well as in humans [16–18]. In the rat model, HTLV-1 provirus was detected in at least 22% of the offspring from a female rat that had been infected with HTLV-1 by intravenous inoculation with MT-2, an HTLV-1-producing human T-cell line [16]. Furthermore, another study using this rat model demonstrated that MTCT was established mainly through breastfeeding [19]. These reports indicate that this rat model can be a useful tool to investigate HTLV-1 MTCT, although the rate of transmission does not appear to be high.

It is well known that cell-to-cell contact is essential for efficient HTLV-1 transmission [20]. The HTLV-1 envelope consisting of gp46 and gp21 has been reported to play an essential role in viral entry into cells [21]. LAT-27 is a well-characterized mAb against gp46, which originated from a WKA strain inbred rat vaccinated with an HTLV-1 Env-expressing recombinant vaccinia virus [22]. LAT-27 is known to have the ability to directly neutralize HTLV-1 infection and to eradicate infected cells through antibody-dependent cellular cytotoxicity (ADCC) in vitro [23]. Furthermore, a recent study demonstrated that passive immunization of pregnant SD strain inbred rats with LAT-27 completely protected their offspring from de novo intraperitoneal HTLV-1 infection [24], suggesting that maternal neutralizing antibodies may have the ability to protect from systemic HTLV-1 infection in vivo. However, it is still unclear whether neutralizing Abs have the potential to prevent mucosal HTLV-1 infection and natural MTCT in vivo.

We previously demonstrated that oral inoculation of MT-2 in immunocompetent rats established a persistent HTLV-1 infection without anti-HTLV-1 Ab responses [11, 25]. In the present study, we developed an MTCT model with an increased rate of transmission using rats orally inoculated with ILT-M1, an HTLV-1-infected human T cell line. We also confirmed that these infected mother rats did not have anti-HTLV-1 Ab responses. These data indicate that this MTCT model would be useful to investigate not only the mechanisms of MTCT but also the role of anti-HTLV-1 Ab in MTCT. Furthermore, we found that the injection of LAT-27 into an orally infected mother rat did not inhibit MTCT of HTLV-1 when only a small amount of LAT-27 was transferred to the offspring. The pre-treatment of adult rats with LAT-27 inhibited intraperitoneal but not oral HTLV-1 infection, indicating that the protective effect of neutralizing Abs on HTLV-1 infection may differ among the route of infection. Our findings will contribute to understanding the role of maternal anti-HTLV-1 Abs in MTCT of HTLV-1, providing important information that should be taken into consideration upon developing a strategy for protection from MTCT via breastfeeding.

RESULTS

Oral inoculation of rats with ILT-M1 cells established a persistent infection with higher HTLV-1 PVL and no antibody responses to HTLV-1 antigens

In an in vitro study, HTVL-1 transmission from ILT-M1 cells to primary human T cells resulted in more efficient transformation of the primary T cells when compared with transformation by MT-2 cells (Y. Tanaka, University of the Ryukus, personal communication). Therefore, we first generated orally or intraperitoneally HTLV-infected rats using ILT-M1 cells and assessed PVL in each infected rat. As shown in Fig. 1(a), orally infected rats tended to carry higher PVL than intraperitoneally infected rats, which is consistent with our previous study using MT-2 cells [11]. A particle agglutination (PA) assay revealed that no Abs against HTLV-1 antigens were detected in orally infected rats (14A01 to 04), whereas intraperitoneally infected rats had anti-HTLV-1 Abs (11A16 and 18, Fig. 1b). This is also consistent with our previous study showing that intravenous or intraperitoneal, but not oral, inoculation of MT-2 cells in rats induced anti-HTLV-1 Ab responses [25]. As the PA assay was not able to detect Abs against HTLV-1 Env protein (data not shown), we examined whether orally or intraperitoneally infected rats had Abs against HTLV-1 Env gp46 by immunoblot analysis (Fig. 1c). Abs against Gag (p53, p24 and p19) were not present in the sera of orally infected rats (14A01 to 04). In contrast, anti-Gag Abs were induced in intraperitoneally infected rats (11A16 and 18, Fig. 1c). However, anti-gp46 Ab responses were not induced in either orally or intraperitoneally infected rats. These results indicate that oral inoculation of ILT-M1 cells leads to an increase in the number of HTLV-1-infected cells and no induction of anti-HTLV-1 Ab responses in rats.

HTLV-1 was efficiently transmitted to offspring derived from orally infected mother rats

A previous study using F344 strain inbred rats reported that HTLV-1 was transmitted to at least two of nine offspring from a mother rat with intravenous HTLV-1 infection [16]. In humans, a higher HTLV-1 PVL in PBMCs is known to be a significant predictor for the increased risk of MTCT [14]. These reports encourage us to examine how efficiently HTLV-1 could be transmitted to the offspring of orally infected rats because orally infected rats carried a higher PVL (Fig. 1). In this experiment, the infected mother rats fed the offspring breast milk until they spontaneously stopped breastfeeding. The offspring were then separated from their mother and killed at 6–10 weeks after birth. As expected, proviral DNA was detected in 50–100% of the
first offspring born to four orally infected rats (Table 1).

The rate of MTCT (average 70%) was much higher than that described in an intravenously HTLV-1-infected rat (22%, [16]) and humans (10–25%, [26]). One of the mother rats (#14A-2) experienced parturition three times (offspring groups 2-1, -2 and -3, Table 1). A higher rate of MTCT was also detected in the second and third offspring (Group 2-2, 66.7%; and 2-3, 100%; Table 1). Notably, in this model, an elevated PVL of infected mother rats tended to be involved with a higher rate of HTLV-1 transmission (Fig. 2a and Table 1). However, the first litters in groups 1 and 3 showed a higher PVL than those in groups 2-1 and 4 (Fig. 2b). Even among the different litters from the same mother rat (#14A-2), a variable level of PVL was observed (Fig. 2c). These results indicate that PVL of the mother rats may be predictive of the rate of HTLV-1 transmission to offspring rather than PVL of the offspring in this model. These data suggest that this animal model may be a useful

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**Table 1. Detection of HTLV-1 provirus in offspring born to HTLV-1-infected mother rats**

<table>
<thead>
<tr>
<th>Group (n)*</th>
<th>HTLV-1-infected mother rats†</th>
<th>Detection of HTLV-1 provirus§</th>
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<td></td>
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<td>Time of delivery after infection (days)</td>
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<td>178</td>
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<tr>
<td>Group 2-1 (n=10)</td>
<td>14A02</td>
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<td>71</td>
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<td>Group 2-2 (n=6)</td>
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<td>119</td>
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<tr>
<td>Group 2-3 (n=5)</td>
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<td>13.6</td>
<td>158</td>
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</table>

*Each litter of offspring born to an HTLV-1-infected mother rat was categorized as a group. n, the total number of offspring.
†F344 n/+ female rats infected with HTLV-1 by oral inoculation of ILT-M1 were mated with uninfected F344 n/n male rats.
‡The PVL is represented as the copy number of HTLV-1 provirus per 10⁵ splenocytes.
§Number of offspring positive for HTLV-1 provirus in splenocytes by PCR at the time of necropsy/total number of offspring.
¶Number of days between birth and necropsy.
§Number of offspring positive for HTLV-1 provirus in PBMCs by PCR at the time of necropsy/total number of offspring.

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**Fig. 1.** PVL and anti-HTLV-1 antibody responses in intraperitoneally (i.p.) and orally (p.o.) infected rats. (a) The PVL in splenocytes of i.p. and p.o. infected rats was measured by quantitative PCR (qPCR). PVL is represented by the copy number of HTLV-1 per 10⁵ splenocytes. Bars indicate the mean PVL. (b, c) The presence of anti-HTLV-1 antibodies in the sera of two i.p. infected rats (11A16 and 11A18) and four p.o. infected rats (14A01 to 14A04) was assessed using Serodia HTLV-I (b) and Problot HTLV-I (c). (b) Antibodies to HTLV-1 in the serum were detected by reading the agglutination patterns of gelatin particles coated with (sensitized particles, right lane) or without (unsensitized particles, left lane) purified HTLV-1 antigens. The reactive control serum containing rabbit anti-HTLV-1 antibodies was used as a positive control (positive Ctrl). The serum from an uninfected rat was prepared as a negative control (negative Ctrl). (c) The positive control serum provided in the kit was used as a positive control (positive Ctrl). The serum from an uninfected rat was prepared as a negative control (negative Ctrl). LAT-27 was used at a concentration of 100 µg ml⁻¹ (LAT-27).
tool to investigate the mechanisms underlying the MTCT of HTLV-1 and to develop novel vaccines to prevent MTCT.

**LAT-27 inhibited HTLV-1 infection in vitro**

Considering that orally infected rats showed no HTLV-1-specific humoral immune responses (Fig. 1b, c), no HTLV-1-specific Abs can be transferred to the offspring in this MTCT model. This means that this MTCT model may have the potential to elucidate the effect of anti-HTLV-1 maternal Abs on MTCT of HTLV-1. As cell-to-cell contact mediated by HTLV-1 Env is essential for efficient HTLV-1 transmission [21], anti-Env Abs are likely to be the best candidates to analyse the role of anti-HTLV-1 Abs in MTCT of HTLV-1. Among the anti-Env Abs purified to date, LAT-27 (rat IgG2b) is a well-characterized mAb specific for Env gp46, and has the ability to neutralize HTLV-1 infection [22]. We first determined the optimal concentration of LAT-27 to block HTLV-1 Env-mediated cell-to-cell fusion of HTLV-1-infected ILT-M1 with HTLV-1-negative K562 cells, after confirming that LAT-27 could bind to gp46 on the surface of ILT-M1 cells, but WAP-24 (rat IgG2b), an anti-HIV-1 Gag p24 mAb, could not (Fig. 3a). Twenty-four hours of cultivation of ILT-M1 cells with K562 cells in the absence of LAT-27 resulted in the formation of multiple syncytia (arrowheads, Fig. 3b, upper left panel). Such syncytium formation was not inhibited even in the presence of high concentrations of WAP-24, an isotype control for LAT-27 (50 µg/ml, Fig. 3b, upper right panel). In contrast, syncytium formation was profoundly inhibited in the cultures with LAT-27 at 50 and 10 µg/ml (Fig. 3b, lower left and middle panels, respectively). However, addition of 1 µg LAT-27/ml to the culture did not block syncytium formation (Fig. 3b, lower right panel). This result indicates that at least 10 µg LAT-27/ml is required to block cell-to-cell fusion of ILT-M1 cells with K562 cells in vitro.

We further assessed the inhibition of cell-to-cell HTLV-1 transmission by LAT-27 using a sensitive HTLV-1-mediated cell fusion assay with slight modification [27]. ILT-M1 cells were co-cultured for 48 h with K562 cells which had been transfected with an HTLV-1 Tax-inducible luciferase reporter plasmid, HTLV-long terminal repeat (LTR)/pGL4, in the presence of various concentrations of LAT-27 (Fig. 3c). The higher concentrations of LAT-27 (100, 50 and 10 µg/ml) significantly reduced the luciferase activity when compared with the same concentrations of WAP-24. However, increased luciferase activity was observed in the absence of LAT-27.
presence of less than 10 µg LAT-27 ml⁻¹ (5, 1, 0.5 and 0.1 µg ml⁻¹). These data clearly indicate that at least 10 µg LAT-27 ml⁻¹ is required to inhibit gp46-mediated cell-to-cell fusion and HTLV-1 infection in vitro.

**LAT-27 inhibited intraperitoneal but not oral HTLV-1 infection in vivo**

To investigate the role of LAT-27 in primary HTLV-1 infection in vivo, rats were intraperitoneally pretreated with LAT-27 24 h before intraperitoneal HTLV-1 infection (the first treatment) and then divided into two groups for the second Ab treatment at different time points: 5 h pre- or post-infection (Fig. 4a, b, respectively). As shown in Fig. 4(a), rats pretreated with LAT-27 5 h before intraperitoneal HTLV-1 infection showed significantly decreased PVL at 8 weeks post-infection when compared with rats pretreated with WAP-24 (LAT-27 vs WAP-24: 0.4 vs 4.7 copies 10⁻⁵ splenocytes, P=0.0286). A significant reduction of PVL was also observed in rats re-injected with LAT-27 5 h post-infection, even though the difference was not statistically significant (LAT-27 vs WAP-24: 2.6 vs 8.1 copies 10⁻⁵ splenocytes, P=0.0152, Fig. 4b), implying that LAT-27 has the potential to limit primary HTLV-1 infection. Notably, PVL in rats pretreated with LAT-27 5 h pre-infection tended to be lower than that in rats injected with LAT-27 5 h post-infection, even though the difference was not statistically significant (LAT-27 vs WAP-24: 0.4 vs 4.7 copies 10⁻⁵ splenocytes, P=0.0666, Fig. 4a, b). This suggests that the presence of higher concentrations of LAT-27 at the site of HTLV-1 exposure may be required for efficient suppression of primary HTLV-1 infection. Some Abs are known to mediate ADCC, complement-dependent cytotoxicity (CDC) and/or antibody-dependent cell-mediated phagocytosis (ADCP).
Indeed, LAT-27 was also reported to induce the depletion of HTLV-1-infected human T cells by human NK cells [23]. However, we detected very low levels of LAT-27-mediated ADCC between rat NK cells and gp46-expressing HTLV-1-infected rat T cells (data not shown). Furthermore, neither CDC nor ADCP against HTLV-1-infected rat T cells was induced by LAT-27 (data not shown), suggesting that, in our rat model, LAT-27 has the potential to suppress HTLV-1 infection in vivo primarily through its neutralizing activity against HTLV-1.

HTLV-1 is mainly transmitted from mother to child through breastfeeding [12, 28]. In this case, the child is exposed to HTLV-1 at the mucosal surface of the digestive tract. We therefore examined the effect of LAT-27 treatment on oral HTLV-1 infection in our rat model. Unexpectedly, rats treated twice with LAT-27 before oral HTLV-1 infection showed a comparable level of PVL to rats pretreated with WAP-24 (LAT-27 vs WAP-24:20.5 vs 32.6 copies 10⁵ splenocytes, P=0.4857, Fig. 4c). Both Abs against HTLV-1 and HTLV-1 antigens are known to be contained in the breast milk of HTLV-1-infected mothers [29, 30]. In humans, maternal IgG is mainly transferred into the fetal circulation across the placenta. To mimic MTCT in humans, rats were intraperitoneally pretreated with LAT-27, and then infected with HTLV-1 by oral inoculation of HTLV-1-infected ILT-M1 cells together with LAT-27. Even in this setting, the treatment with LAT-27 could not inhibit oral HTLV-1 infection (LAT-27 vs WAP-24:23.1 vs 5.6 copies 10⁵ splenocytes, P=1.0, Fig. 4d). These results

![Image](image_url)
suggest that neutralizing Abs in systemic circulation less effectively prevent oral HTLV-1 infection in our rat model.

**LAT-27 inoculated into orally infected mother rats was transferred to their offspring but did not inhibit mother-to-child HTLV-1 transmission**

We next evaluated whether passive transfer of LAT-27 into an orally infected mother rat prevented the mother-to-child HTLV-1 transmission. An orally infected rat (15R02) was treated with LAT-27 (10 mg, intraperitoneally) before being pregnant, and gave birth to the first litter (n=6, group 6-1) and the second litter (n=13, group 6-2) at 53 and 123 days after Ab treatment, respectively (Table 2). Unexpectedly, all of the six offspring in group 6-1 were found to have HTLV-1 provirus in PBMCs at 4 weeks after birth, although the provirus was detectable in splenocytes of only 50% of the offspring (Fig. S1a, available in the online Supplementary Material). In group 6-2, HTLV-1 provirus in PBMCs or splenocytes was detected in five or six out of 13 offspring, respectively (PBMCs vs splenocytes; 38.5% vs 46.2%, Table 2 and Fig. S1a). Intriguingly, PVL in group 6-2 was significantly lower in PBMCs but not splenocytes than that in group 6-1 (group 6-1 vs group 6-2: 1870 vs 1.9 copies 10⁻⁵ PBMCs, P=0.0043, Fig. 5a, 0.12 vs 0.11 copies 10⁻⁵ splenocytes, P=0.8496, Fig. S1b). Furthermore, in all groups, PVL in PBMCs tended to be higher than that in splenocytes (Figs 5a and S1b). A lower concentration of maternal LAT-27 was transferred to all offspring in group 6-1. Because, in rats, maternal IgG is transferred to the offspring mainly through breast milk, this result indicates that LAT-27 persisted in the mother rat at least until 53 days after LAT-27 injection even though the half-life of serum LAT-27 was around 14 days (Table 3). In contrast, LAT-27 was not detectable in any offspring in group 6-2 (Table 2 and Fig. 5b). These results suggest that the maternal neutralizing Ab may not prevent milk-borne HTLV-1 transmission to the offspring when a low titre of the Ab is transferred from infected mothers.

**DISCUSSION**

HTLV-1 infection during childhood is believed to be a risk factor for the development of ATL [9]. However, prophylactic drugs and vaccines to prevent MTCT of HTLV-1 have not yet been developed because the mechanisms of MTCT are largely unknown. Therefore, not only human studies but also in vivo studies using animal models are required for analysis and prevention of MTCT of HTLV-1. In this study, we demonstrate a rat model of natural MTCT using orally HTLV-1-infected rats showing no Ab responses against HTLV-1.

To date, natural MTCT of HTLV-1 has been reported in non-inbred Japanese white rabbits and F344 strain inbred rats. In rabbits, HTLV-1-infected female rabbits transmitted HTLV-1 infection to four of seven (57.1%) offspring [31]. In F344 rats, HTLV-1 was transmitted to two of nine (22.2%) offspring born to a mother rat that had been intravenously inoculated with MT-2 cells [16]. Those animal models are useful for analysis and prevention of MTCT of HTLV-1. However, rabbits are less convenient for use as laboratory animals than rats, and there is a paucity of commercially available immunological reagents for the rabbit, even though a higher rate of HTLV-1 transmission can be obtained. In the rat model of MTCT, the rate of natural MTCT of HTLV-1 does not appear to be high. Given that a higher PVL of HTLV-1-infected mothers is a significant predictor of the increased risk of MTCT in humans, in this study we used orally infected mother rats in our MTCT model rather than intraperitoneally infected rats because orally infected rats showed a relatively higher PVL (Fig. 1a). As expected, this MTCT model showed a higher rate of MTCT ranging from 50 to 100% (average 70%, Table 1). Furthermore, PVL of infected mother rats was probably correlated to the rate of HTLV-1 transmission to the offspring (Fig. 2a), but not with PVL of the offspring (Fig. 2b), which is consistent with previous reports [13, 14, 32]. These results suggest that PVL of infected mothers may be one of the determinants of the risk of MTCT in rats as well as in humans.

In this study, we first used splenocyte samples of the offspring at 6–10 weeks after birth to determine PVL (Table 1, Fig. 2b, c). However, in the offspring at 4 weeks after birth, the PVL was higher in PBMCs than in splenocytes (Figs 5a and S1b). Furthermore, HTLV-1 provirus in splenocytes was detectable in only 50% of the offspring in

<table>
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<th>Group (n)</th>
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<td>Group 6-2 (n=13)</td>
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<td>LAT-27</td>
<td>31</td>
<td>123</td>
<td>5/13</td>
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</table>

*Each litter of offspring born to an Ab-treated HTLV-1-infected mother rat was categorized as a group. n, Total number of offspring.†F344 n/+ female rats infected with HTLV-1 by oral inoculation of ILT-M1 were mated with uninfected F344 n/n male rats.‡HTLV-1-infected mother rats were intraperitoneally injected with 10 mg WAP-24 or LAT-27 before or during the period of pregnancy.§Number of offspring positive for HTLV-1 provirus in PBMCs by PCR at the time of necropsy/total number of offspring.
group 6-1 (Fig. S1a), even though all of the offspring had infected cells in PBMCs (Table 2). In the group 2-1 offspring shown in Table 1 and Fig. 2, the PVL in PBMCs reached a peak at 4–5 weeks after birth and then declined to a stable HTLV-1 set point at 6 weeks. At 6 weeks after birth and thereafter, the level of PVL in PBMCs of group 2-1 was comparable to that in splenocytes at 10 weeks (data not shown). Indeed, in group 2-1, the PVL in PBMCs at 4 weeks after birth tended to be higher than that in splenocytes at 10 weeks (Fig. S1c). These results suggest that, in the offspring at 4 weeks after birth, PBMCs would be a good sample to sensitively evaluate MTCT of HTLV-1. They also suggest that HTLV-1-infected cells in peripheral blood may be gradually distributed in peripheral lymphoid tissues, such as spleen and lymph nodes, around 4–6 weeks after birth. It is also possible that HTLV-1-specific T-cell responses are induced in the offspring by HTLV-1 infection and contribute to reduction of PVL around 4–6 weeks after birth. In the present study, we did not analyse HTLV-1-specific T-cell responses in the offspring. Further studies are required to clarify the reasons for reduction of PVL in the circulation around 4–6 weeks after birth.

It has been suggested that a higher titre of anti-HTLV-1 Abs in infected mothers is associated with an increased risk of vertical transmission of HTLV-1 to children [15]. In contrast, it was also reported that milk-borne transmission of HTLV-1 from infected mother rabbits to their offspring was prevented by passive immunization of anti-HTLV-1 Abs to the offspring [33], indicating the protective effect of anti-HTLV-1 Abs on MTCT. Due to these contradictory findings, the role of anti-HTLV-1 Abs in MTCT remains unclear. In this study, consistent with our previous studies using MT-2 cells as a source of HTLV-1 infection [11, 25], oral inoculation of ILT-M1 cells in adult rats induced no Ab responses against HTLV-1 antigens, such as Gag and Env (Fig. 1b, c), indicating that no maternal Abs against HTLV-1 should be transferred from orally infected mother rats to their offspring. This result suggests that this MTCT model can be a useful tool to evaluate the effect of an Ab on MTCT by means of passive immunization with the Ab to orally infected mother rats.

LAT-27, an mAb against HTLV-1 Env gp46, has been reported to neutralize cell-to-cell HTLV-1 transmission from HTLV-1-infected cells to uninfected cells [22]. A recent study demonstrated that de novo intraperitoneal HTLV-1 infection was completely suppressed in the offspring born to pregnant rats that had been passively immunized with LAT-27 [24], suggesting that LAT-27 in the mother rat can be transferred to her offspring and has the

### Table 3. Serum concentration of LAT-27 after intraperitoneal injection

<table>
<thead>
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*Two uninfected rats were intraperitoneally inoculated with LAT-27 (10 mg).
†Serum LAT-27 concentration was quantified by ELISA.
‡The fold change was calculated by dividing the concentration of LAT-27 at the indicated time points by that at day 1 after LAT-27 injection.
potential to prevent HTLV-1 infection in her offspring. In contrast, in the present study, passive immunization of an infected mother rat with LAT-27 did not suppress the rate of HTLV-1 transmission to the first litter of offspring (Group 6-1: 100%, Table 2). These offspring were found to have a small amount of LAT-27 in their sera (Group 6-1: average 0.5 µg ml⁻¹, Fig. 5b). Considering our finding that at least 10 µg LAT-27 ml⁻¹ was required for inhibition of cell-to-cell HTLV-1 transmission between HTLV-1-infected and uninfected cells (Fig. 3b, c), the transfer of only a small amount of LAT-27 to the first litter may be one of the reasons why the rate of HTLV-1 MTCT was not reduced. Intriguingly, the rate of MTCT of HTLV-1 was reduced in the second litter born to the same infected mother rat (Group 6-2: 38.5%, Table 2) even though an undetectable level of LAT-27 was transferred to the offspring (Group 6-2: undetectable, Fig. 5b). This finding raises two possibilities concerning the role of LAT-27 in MTCT. First, LAT-27 gradually inhibits/eliminates HTLV-1-infected cells in the infected mother. In this case, the second litter would be exposed to a smaller number of HTLV-1-infected cells than the first litter. Second, a small amount of LAT-27 may enhance HTLV-1 infection. It has been reported that a low to moderate titre of maternal neutralizing Abs (IgG) against cytomegalovirus (CMV) causes neonatal Fc receptor-mediated virion transcytosis at the uterine–placenta interface, leading to the enhancement of vertical CMV infection. In contrast, a higher titre of maternal IgG to CMV inhibits vertical CMV infection [34]. Transcytosis of human immunodeficiency virus 1 (HIV-1) viral is also enhanced by Abs in vitro [35]. Therefore, it is possible that a small amount of LAT-27 (IgG2b) promoted HTLV-1 transmission to the first litter. However, we did not determine PVL of the infected mother rat at the two time points when she delivered the first and second litters. In addition, we performed this experiment using only one infected mother rat. Further studies with several infected mother rats are required to elucidate the role of maternal neutralizing Abs in MTCT of HTLV-1.

In adult rats intraperitoneally pretreated with LAT-27, intraperitoneal HTLV-1 infection was significantly inhibited (Fig. 4a). Intraperitoneal injection of LAT-27 before and after intraperitoneal HTLV-1 infection also reduced PVL (Fig. 4b), indicating that LAT-27 has the potential to prevent primary HTLV-1 infection in vivo. Furthermore, PVL of adult rats pretreated twice with LAT-27 before HTLV-1 infection was found to be lower than that of rats treated before and after infection (Fig. 4a, b), suggesting that the presence of a higher amount of LAT-27 at the exposure site of HTLV-1 may be required to efficiently suppress HTLV-1 infection. However, oral HTLV-1 infection was not suppressed by intraperitoneal pretreatment with LAT-27 in adult rats (Fig. 4c), despite a high level of serum LAT-27 (Table 3), indicating that it may be difficult to redistribute LAT-27 from the circulation to the lumen of the digestive tract. These results suggest that the neutralizing activity of LAT-27 at the exposure site of HTLV-1 primarily contributes to protection from HTLV-1 infection. Maternal Abs against HTLV-1 have been shown to be transferred to the offspring mainly through breast milk in rats [36]. It has also been suggested that breast milk of infected mothers contains HTLV-1-infected cells in rats as well as in humans [16, 17, 19, 37, 38]. Thus, both HTLV-1-infected cells and anti-HTLV-1 Abs are contained in the breast milk of infected mothers. Unexpectedly, oral inoculation of LAT-27 in conjunction with HTLV-1-infected cells did not inhibit HTLV-1 infection in adult rats that had been intraperitoneally pretreated with LAT-27 (Fig. 4d). Several mechanisms underlying MTCT of HTLV-1 were suggested. First, HTLV-1-infected lymphocytes may cross the mucosal barriers between enterocytes and/or through M cells [39]. Second, enterocytes are susceptible to HTLV-1 infection in vitro [40]. Finally, HTLV-1 virions can pass through epithelial cells by transcytosis [41]. Therefore, some of these mechanisms may explain why mucosal HTLV-1 infection was not inhibited even in the presence of a high titre of LAT-27 both in the circulation and at the mucosal surface of the digestive tract (Fig. 4d). It is also possible that low pH in the stomach of adult rats dissociates immune complexes between HTLV-1-infected cells and LAT-27, and degrades LAT-27 when it passes through the stomach. Further studies are required to understand the role of anti-HTLV-1 Abs in mucosal HTLV-1 infection.

To prevent MTCT of HTLV-1, bottle-feeding of formula milk is recommended because it has been shown that cessation of breast milk feeding reduces the risk of MTCT of HTLV-1 [42, 43]. However, the interruption of breastfeeding affects not only the economics of the family and the community but also individual and public health, such as malnutrition and increased mortality of infants, and increased risk of breast and ovarian cancer of the mothers. Furthermore, the development of bonding and attachment between the mother and the infant is hindered without breastfeeding. The infected mothers are also advised to bottle-feed freeze–thawed breast milk because the freeze–thawing method eliminates HTLV-1-infected cells in breast milk, resulting in a reduced risk of MTCT [43, 44]. However, this is a laborious task and probably impractical for many infected mothers. Therefore, in Japan, breastfeeding for a maximum of 3 months is also an option, as studies with small Japanese sample sizes suggest that a reduction in MTCT by short-term breastfeeding (<3 months) was comparable to that by bottle-feeding. However, it seems to be difficult for some infected mothers to stop breastfeeding at 3 months after birth of the infant. It is of note that all three methods described above are not able to completely prevent MTCT although they effectively reduce the risk of MTCT. Therefore, establishment of vaccine strategies to reduce PVL in breast milk and PBMCs of infected mothers would be helpful to further reduce the risk of MTCT.

A recent report demonstrated that passive immunization of a humanized LAT-27 Ab to humanized mice completely prevented primary HTLV-1 infection through...
neutralization and ADCC mediated by the Ab [24], indicating the importance of a humanized Ab-mediated ADCC as well as neutralization for the efficacy of immunotherapies with neutralizing Abs. In this study, passive immunization with LAT-27 did not limit mucosal HTLV-1 infection in our rat models, which may in part be due to only a low induction of ADCC mediated by LAT-27. Further studies using Abs capable of inducing strong ADCC will be required for the development of a passive immunization strategy to effectively prevent HTLV-1 MTCT.

In this study, we developed a new rat model of natural HTLV-1 MTCT with no maternal Abs against HTLV-1. This model will be a useful tool to analyse the role of maternal Abs in MTCT of HTLV-1. Furthermore, we also demonstrated that a neutralizing Ab had the ability to inhibit HTLV-1 infection in vivo. However, its protective efficiency may vary depending on the route of infection. Our findings will contribute to understanding the role of maternal Abs in MTCT of HTLV-1, providing important information to be considered upon designing a novel strategy to prevent MTCT of HTLV-1 in humans.

METHODS

Animals

Four-week-old female F344/NJcl-rnu/+ (F344 n/+ and male F344/NJcl-rnu/rnu (F344 n/n) rats were purchased from CLEA Japan. All rats were maintained in sterile cages within sterile filter hoods, and fed sterilized food and water at the BSL3 area of the Center for Experimental Animals in the Tokyo Medical and Dental University. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University. All treatments, such as HTLV-1 infection and Ab injection, were performed under isoflurane anaesthesia.

Cell lines

The human erythroleukaemic cell line K562 was cultured in RPMI 1640 medium (Gibco Laboratories) supplemented with 10% FBS (Sigma Aldrich), 100 IU penicillin ml⁻¹, 100 µg streptomycin ml⁻¹ and 2 mg sodium bicarbonate ml⁻¹. An IL-2-dependent HTLV-1-infected T-cell line derived from an HAM/TSP patient, ILT-M1 [45], was maintained in RPMI 1640 medium containing 20% FBS and 30 U recombinant human IL-2 ml⁻¹ (rhIL-2; Shionogi Pharmaceutical).

Antibodies

The Abs used in this study were LAT-27 (rat IgG2b), an anti-HTLV-1 Env gp46 neutralizing mAb [22], and WAP-24 (rat IgG2b), an anti-HIV-1 Gag p24 mAb [46] was used as an isotype control Ab for LAT-27.

HTLV-1 infection

Five-week-old F344 n/+ rats were infected with HTLV-1 by oral or intraperitoneal inoculation of ILT-M1 cells (2×10⁶ cells). In brief, for oral infection, ILT-M1 cells in 0.5 ml of PBS were directly administered into the oesophagus through a feeder tube. For intraperitoneal infection, ILT-M1 cells were percutaneously injected into the abdominal cavity.

Effect of LAT-27 on primary HTLV-1 infection

Five-week-old F344 n/+ rats were i.p. injected with LAT-27 (1 mg per injection) at 24 and 5 h before intraperitoneal or oral HTLV-1 infection. In another experiment, LAT-27 (1 mg per injection) was intraperitoneally injected into 5-week-old F344 n/+ rats at 24 h before and 5 h after intraperitoneal HTLV-1 infection. At 8 weeks after infection, splenocytes were collected from these rats to quantify HTLV-1 provirus.

Kinetics of serum LAT-27 in rats

Five-week-old F344 n/+ rats were intraperitoneally inoculated with LAT-27 (10 mg). Peripheral blood samples were collected at various time points, and the levels of LAT-27 in the sera were determined by ELISA.

Mother-to-child HTLV-1 transmission

Five-week-old female F344 n/+ rats were orally infected with HTLV-1, and mated 4 weeks later with HTLV-1-uninfected male F344 n/n rats. After parturition, the infected mother rats breast-fed their offspring. Four weeks later, the offspring were weaned and transferred out of the mother’s cage. At 6–10 weeks after birth, splenocytes and PBMCs were collected to detect HTLV-1 provirus. In another experiment, the infected female rats were intraperitoneally inoculated with LAT-27 (10 mg) before pregnancy. The offspring were breast-fed by their mother until weaning. At 4 weeks after birth, peripheral blood and spleen were collected from the offspring to quantify HTLV-1 provirus and determine the levels of maternal LAT-27 in the sera.

Flow cytometry

ILT-M1 cells (2.5×10⁶ cells) were incubated with (10 µg ml⁻¹) LAT-27, WAP-24 or purified rat IgG2b Ab (clone RTK4530; BioLegend) for 15 min at room temperature. The cells were then washed and stained with PE-conjugated goat anti-rat IgG Ab at 2 µg ml⁻¹ (clone; Poly4054; BioLegend). Fifteen minutes later, the cells were washed and fixed in 1% formaldehyde. Samples were analysed on a FACSCalibur (BD Biosciences), and data analyses were performed using FlowJo software (Tree Star).

Syncytium inhibition assay

ILT-M1 cells (5×10⁵ cells) were pretreated with or without LAT-27 or WAP-24 at 2, 20 or 100 µg ml⁻¹ at 37°C for 15 min and then co-cultured with K562 cells (1×10⁵ cells) at 37°C for 24 h. The cells were observed under an optical microscope (magnification, 100×).

Luciferase reporter assay

The fragment comprising a concatenator of 21 bp Tax-inducible cyclic AMP responsive elements (CREs) of HTLV-1 LTR and an enhancerless HTLV-1 promoter was subcloned from WT-Luc plasmid [47] into pGL4.17 [luc2/Neo] plasmid (Promega) to generate HTLV-LTR/pGL4.
K562 cells (2.5 × 10⁵ cells) electrically transfected with HTLV-LTR/pGL4 were co-cultured with mitomycin C-treated ILT-M1 cells (1.25 × 10⁶ cells) in the presence or absence of various amounts of LAT-27 for 48 h. The luciferase activity was measured using the Luciferase Assay System (Promega) and the Lumat LB 9507 luminometer (Berthold Technologies).

**Quantification of HTLV-1 proviral load**

To quantify the HTLV-1 PVL in PBMCs and splenocytes, genomic DNA samples were prepared by SDS–proteinase K digestion, followed by phenol–chloroform extraction. PVL was quantified by real-time PCR on a LightCycler PCR Instrument (Roche Diagnostics). The PCR was performed with the QuantiTect SYBR Green PCR kit (Qiagen) in accordance with the manufacturer’s instructions. Briefly, 20 µl of a PCR mixture containing the following reagents was prepared: genomic DNA (0.5–1 µg), 1× QuantiTect SYBR Green PCR Master Mix and HTLV-1-specific primers (0.5 µM, pX2: 5’-CGGATACCCAGTCTACGTGTTGGAGACTGT-3’ and pX3: 5’-GACGCGAATACCGGTCATCGATGGGTCC-3’) or β-actin-specific primers (0.5 µM, sense: 5’-CTGTATGCGCTCTGTCGA-3’ and antisense: 5’-CCATGCTTGCTGAAACCT-3’). The PCR mixture was subjected to 55 cycles of denaturation (94°C, 15 s), annealing (55°C, 20 s), extension (72°C, 10 s) and denaturation of primer-dimers (82°C, 5 s) following an initial Taq polymerase activation step (95°C, 15 min). The copy numbers of HTLV-1 provirus in the samples were estimated from the standard regression curve with the LightCycler Software version 4.1 (Roche Diagnostics). The standard curve for HTLV-1 provirus was obtained by PCR data for 2 × 10⁵ to 2 × 10⁶ copies of pGEM-pX2-3 plasmid. The pGEM-pX2-3 plasmid was constructed by inserting a PCR fragment amplified with pX2 and pX3 from the genomic DNA of MT-2 cells, an HTLV-1-infected human T-cell line, into the pGEM-T Easy vector (Promega). The PVL was calculated as follows: [(copy number of pX)/([copy number of β-actin]) × (2 × 10⁶)], and represented as the number of HTLV-1 copies per 10⁶ PBMCs or splenocytes.

**ELISA**

The titres of LAT-27 in the sera of LAT-27-treated rats and offspring born to LAT-27-treated infected mother rats were measured by ELISA. Briefly, HTLV-1 gp46 synthetic peptide (residues 186–199; TAPPILPHSNLDDLHI) was coated onto 96-well flat-bottom plates (Nunc). LAT-27 binding to the synthetic peptide was detected by HRP-conjugated goat anti-rat IgG Ab (clone; Poly4054; BioLegend). The absorbance was determined at 450 nm using the iMark Microplate Absorbance Reader (BioRad).

**Detection of anti-HTLV-1 antibodies**

The presence of Abs against HTLV-1 antigens in the sera of infected rats was determined by the PA method using Sordia HTLV-I (Fuji Rebio). The Abs specific for HTLV-1 Gag (p53, p24 and p19) and Env gp46 in the rat sera were detected by immunoblotting using Problot HTLV-I (Fuji Rebio). The biotin-conjugated anti-rat IgG (H and L) Ab (clone; ab6733; Abcam) was used as a secondary Ab.

**Statistical analysis**

The Mann–Whitney U-test and the unpaired t-test were performed to test for statistical significance between groups using Graphpad Prism software (Graphpad Software). In all cases, two-tailed P values less than 0.05 were considered statistically significant.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University (No.170004).

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