(IFN) response [19]. It is therefore plausible that the observed small number of Tax-expressing cells after ex vivo culture is likely due to co-culture with mouse stromal cells derived from the spleen. Meanwhile, we observed that the percentage of Tax-expressing cells in the same culture conditions varies from one patient to another even in HAM/TSP patients with similar PVL (Saito et al., unpublished data). Furthermore, the severely immune-deficient NOG mice used in this study do not have any acquired immune response against inoculated HTLV-1 infected cells, such as HTLV-1-specific Abs, helper T cells, and cytotoxic T lymphocytes. These observations suggest that not only culture conditions but also cellular factors might be involved in the number of Tax-expressing cells. It also needs to be clarified whether the small number of Tax-expressing cells can be explained by multiple infection of single cell. Further investigations of such factors would be important for controlling HTLV-1 infection and disease development in vivo.

It is well established that the HTLV-1 virions are not very infectious, and thus cell-to-cell transmission is more efficient both *in vivo* and *in vitro* [20,21]. The surface glycoproteins of HTLV-1, which are recognized by neutralizing antibodies, play important roles in cell-to-cell transmission [22,23]. Indeed, previous reports have indicated that passive transfer of HTLV-1 Env-specific-neutralizing antibodies is effective in preventing *in vivo* infection in macaques [5,24] and rabbit [25,26] models. However, these studies evaluated the in vivo transmission of HTLV-1 to non-human cells, which are more resistant to HTLV-1 infection than human cells are. In this study, we tested the protective efficacy of various anti-HTLV-1 antibodies against HTLV-1 transmission into human lymphocytes *in vivo* in the hu-PBMC-NOG-spl mouse model. The mice immunized with the anti-HTLV-1 gp46 neutralizing mAb (clone LAT-27) were completely protected against HTLV-1 infection whereas other non-neutralizing antibodies such as anti-gp46 mAb (clone LAT-25), anti-Gag (clone GIN-7), anti-HCV (clone MO-8), and anti-OX40 mAb (clone B-7B5) did not protect against infection (Figure 3A). The HTLV-1 proviral DNA was not detected by quantitative real-time PCR in the human lymphocytes recovered from hu-PBMC-NOG-spl mice that received passive transfer of LAT-27, indicating that the neutralizing function is an essential factor in preventing in vivo HTLV-1 transmission. Furthermore, passive immunization with human polyclonal anti-HTLV-1 IgG from HAM/TSP patients (HAM-IgG) can also protect against HTLV-1 infection in vivo, whereas human immunoglobulin isolated from HTLV-1-negative donors (NC-IgG) did not (Figure 3A). Consistent with the results of the quantitative real-time PCR, FCM studies also showed that the human CD4-positive cells recovered from mouse spleens immunized with either LAT-27 or HAM-IgG, express only trace amounts of Tax protein after short-term (16 h) cultivation *ex vivo*, which may be the result of background false-positive staining. In contrast, a significant amount of Tax protein was expressed in human lymphocytes recovered from non-immunized mouse spleens (PBS-injected) or mouse spleens immunized with NC-IgG (Figure 3B). These results demonstrate the requirement for the neutralizing function of the anti-HTLV-1 antibody in preventing *in vivo* transmission. It is noteworthy that neutralizing anti-Env gp46 clone LAT-27 and HAM-IgG completely blocked the in vivo transmission of HTLV-1 in human lymphocytes, even in the conditions that permit the vigorous proliferation of human lymphocytes that enables HTLV-1 to rapidly spread by cell-to-cell contact. However, antibody injection only once after PBMC transplantation did not block the HTLV-1 infection in vivo, suggesting that the pre-existing neutralizing anti-Env Abs are critical for preventing HTLV-1 infection (Additional file 3: Figure S2). This result also suggests that *in vivo* transmission is established within 24 hours after transfer of HTLV-1-infected cells. Importantly, although neutralizing Abs used in this study displayed antibody-dependent cell-mediated cytotoxicity (ADCC) activity in vitro in our previous study [27], such neutralizing and ADCC activities of anti-Env Abs are not crucial for the elimination of HTLV-1-infected cells once HTLV-1 infection is established *in vivo*. Indeed, titers of existing neutralizing and ADCC Abs did not correlate with HTLV-1 PVL (i.e., numbers of HTLV-1-infected cells in vivo) (Saito et al., unpublished data). Moreover, HAM/TSP patients also showed high titers of such Abs, indicating that these Abs are not potent in preventing the onset of HAM/TSP in infected individuals. These data also indicate the importance of passive immunization before infection.

Recently, we reported that both LAT-27 and HAM-IgG, but not non-neutralizing LAT-25 and NC-IgG, are capable of depleting and/or eliminating HTLV-1-infected cells in the presence of autologous PBMCs in vitro. This occurs in part via ADCC, preventing the spontaneous immortalization of T cells [27]. Thus, the neutralizing activity is essential for preventing HTLV-1 infection as well as malignant transformation. In the present study, although non-neutralizing anti-Env gp46 (clone LAT-25) and anti-Gag p19 (clone GIN-7), as well as control antibodies (anti-HCV clone MO-8, anti-OX40 clone B-7B5), and normal human IgG (NC-IgG) did not completely block the infection, we observed that non-neutralizing LAT-25 mAb and anti-OX40 mAb decreased the number of HTLV-1 infected cells to some extent (Figure 3A). Since OX40 is a cell-surface molecule specifically expressed on HTLV-1-infected and activated T cells [28], and LAT-25 recognizes the HTLV-1 Env protein, these data may suggest a novel effect of IgG specifically reacting with a

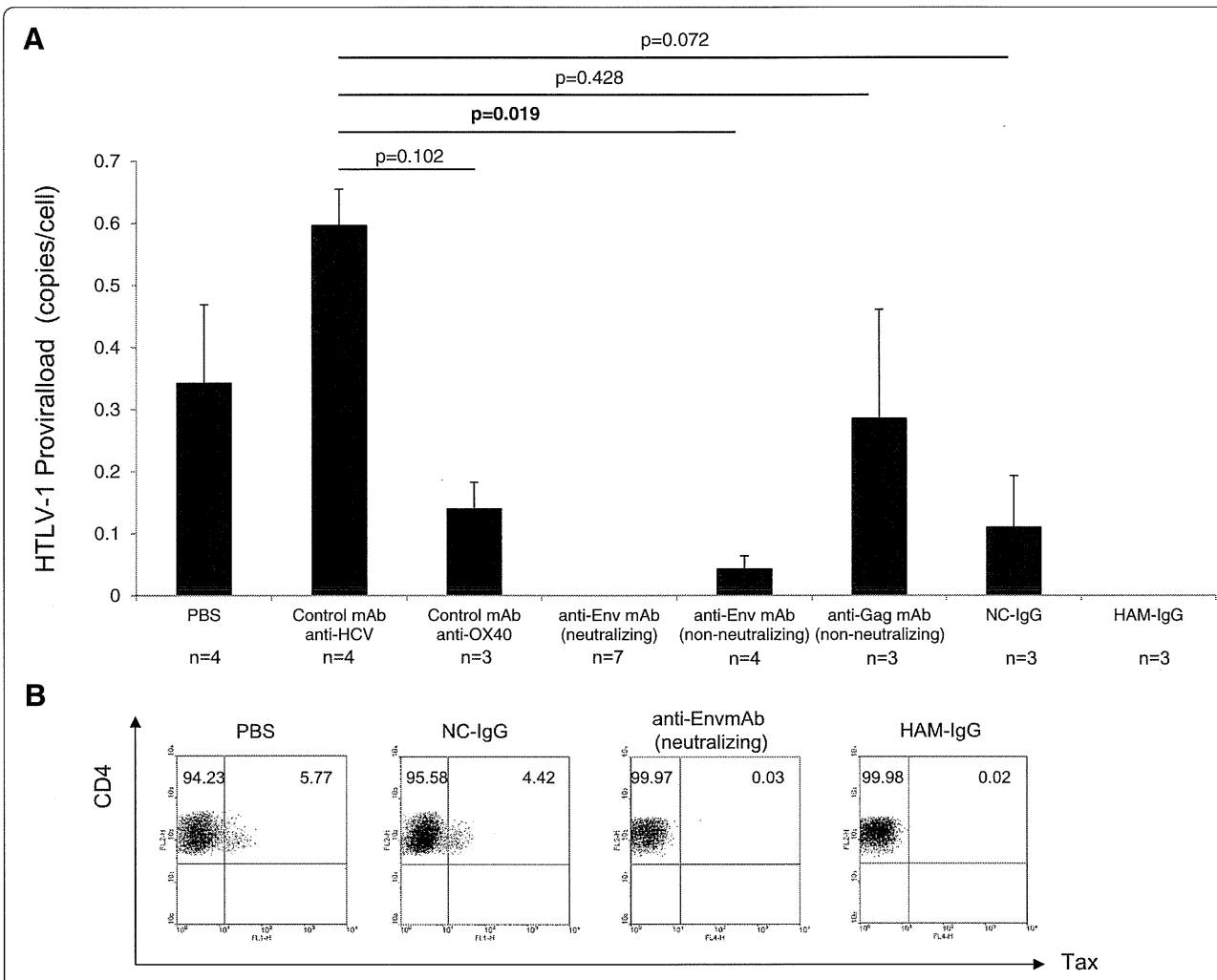

Figure 3 HTLV-1 infection in hu-PBMC-NOG-spl mice was completely inhibited by neutralizing, but not non-neutralizing, antibodies. *In vivo* transmission of HTLV-1 and protective efficacy of various monoclonal antibodies was evaluated using quantitative real-time PCR analysis of HTLV-1 proviral DNA. Genomic DNA was extracted from the human lymphocytes recovered from hu-PBMC-NOG-spl mice. **A**. All of the mice immunized with neutralizing mAbs against Env (clone LAT-27) were completely protected against HTLV-1 infection, whereas non-neutralizing mAbs against Env (clone LAT-25), anti-Gag (clone GIN-7), anti-HCV (clone MO-8), or anti-OX40 mAb (clone B-7B5) did not protect against infection. The mice immunized with human immunoglobulin isolated from HAM/TSP patients (HAM-IgG) were also protected against HTLV-1 infection, whereas human IgG isolated from normal uninfected controls (NC-IgG) did not protect against infection. Results are shown as mean ± SE. To test for significant differences among the different groups, one-way analysis of variance was performed, followed by Scheffe's multiple comparisons test. The lower limit of detection was one copy of HTLV-1 tax per 10^4 PBMCs. **B**. Flow cytometric studies indicated that the human lymphocytes recovered from mouse spleens immunized with anti-Env neutralizing mAbs or HAM-IgG express only a trace amount of Tax protein after short-term (16 h) cultivation *ex vivo*, which may be a background false-positive staining artifact. In contrast, a significant amount of Tax protein was expressed in human lymphocytes recovered from non-immunized mouse spleens (PBS-injected) or mouse spleens immunized with NC-IgG. The numbers represent the percentage of the cell population within the HLA-class I-positive/CD4-positive gate.

number of membrane receptors on HTLV-1-infected and/or activated T cells *in vivo*. As shown in Figure 3A, human IgGs isolated from uninfected people also suppressed the PVL, indicating that the administered non-specific IgGs also can help to eliminate the inoculated HTLV-1-infected cells (i.e., ILT-M1 cells). It is well established that the intravenous immunoglobulins (IVIg) therapy is effective in various diseases including autoimmune diseases and life-threatening infections. Although the precise mechanism of action of injected IVIg is not fully understood, several pathophysiological mechanisms such as suppression of idiotypic antibodies, saturation of Fc receptors on macrophages, modulation of complement activation, and suppression of various immunomodulators such as cytokines, chemokines, and metalloproteinases have been proposed [29]. It is therefore possible that the administered non-specific immunoglobulin in our mouse model also can help to eliminate the inoculated HTLV-1-infected

cells (i.e. ILT-M1 cells) via unknown mechanisms, resulting in a decreased efficiency of *in vivo* infection.

In conclusion, we have established a novel and simple small animal model to study primary HTLV-1 infection in vivo. Although our mouse model is not the animal models of HAM/TSP or ATL, the present study has demonstrated an important rational basis for passive immunization against HTLV-1 infection in humans. Using our mouse model, *in vivo* evaluation of the efficacy of drug candidates could also be investigated in future studies.

Additional files

Additional file 1: Table S1. Sequences flanking the integration site of HTLV-1 provirus in ILT-M1 cell and primer sequences used for integration site-specific PCR.

Additional file 2: Figure S1. To rule out the possible contamination of residual ILT-M1 cells, inverse PCR amplification was carried out to determine the sequences adjacent to HTLV-1 LTRs (both 3′- and 5′-LTR) using the DNA extracted from ILT-M1 cells, as previously described [13]. Then, integration site-specific PCR was carried out using primer pairs that encompass HTLV-1 LTRs (both 3′ and 5′ LTR) and flanking host sequences (Additional file 1: Table S1). As shown, no integration site specific bands were observed except for ILT-M1 cells, suggesting that the possible contamination of HTLV-1 genome derived from the residual ILT-M1 cells is unlikely.

Additional file 3: Figure S2. Flow cytometric studies showed that the human lymphocytes recovered from mouse spleens express the amount of Tax protein after short-term (16 h) cultivation ex vivo, indicating that the neutralizing anti-Env Ab (clone LAT-27) injection once after PBMC transplantation did not block the in vivo transmission of HTLV-1.

Abbreviations

HTLV-1: Human T-cell leukemia virus type-1; ATL: Adult T-cell leukemia; HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis; ADCC: Antibody-dependent cellular cytotoxicity; PVL: Proviral load.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS designed and performed the experiments, analyzed the data, and wrote the paper; RT, HF, AK and YT performed experiments, analyzed and interpreted data; TM and HT provided clinical samples and assembled clinical database; YT made contribution to the conception and design of the study, and wrote the paper. All authors read and approved the final manuscript.

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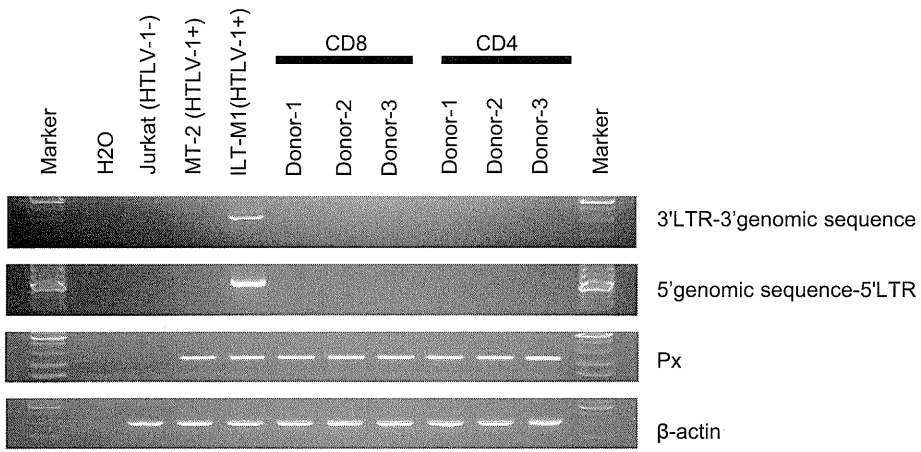
Supplemental Table 1

Sequences flanking the integration site of HTLV-1 provirus in ILT-M1 cell and primer sequences used for integration site-specific PCR.

5'-genomic region	5'-LTR	3'-LTR	3'-genomic region
TGCTTTGTCATCTGTGCGTTCAGTTCA	**TGACAATGACCATGAGCCCCAAATATC**	**TCCAGGAGAGAAACTTAGTACACA**	AGTTCACAGAGTTTCACCTTTCTCTTCA
Forward Primer for the 5'-genomic region	Reverse Primer for the 5'-LTR	Forward Primer for the 3'-LTR	Reverse Primer for the 3'-genomic region
5'-TGCAGATTTCAAGCGCTTCTAGG-3'	5'-TTAGTCTGGGCCCTGACCTTTTCA-3'	5'-CAACTCTACGTCTTTGTTTCGT-3'	5'-GTAAATGAGAAATCCCGCTTCCA-3'

Flanking sequences of 5'- and 3'-LTR were determined by inverse-PCR.
HTLV-1 proviral sequences are shown in boldface.



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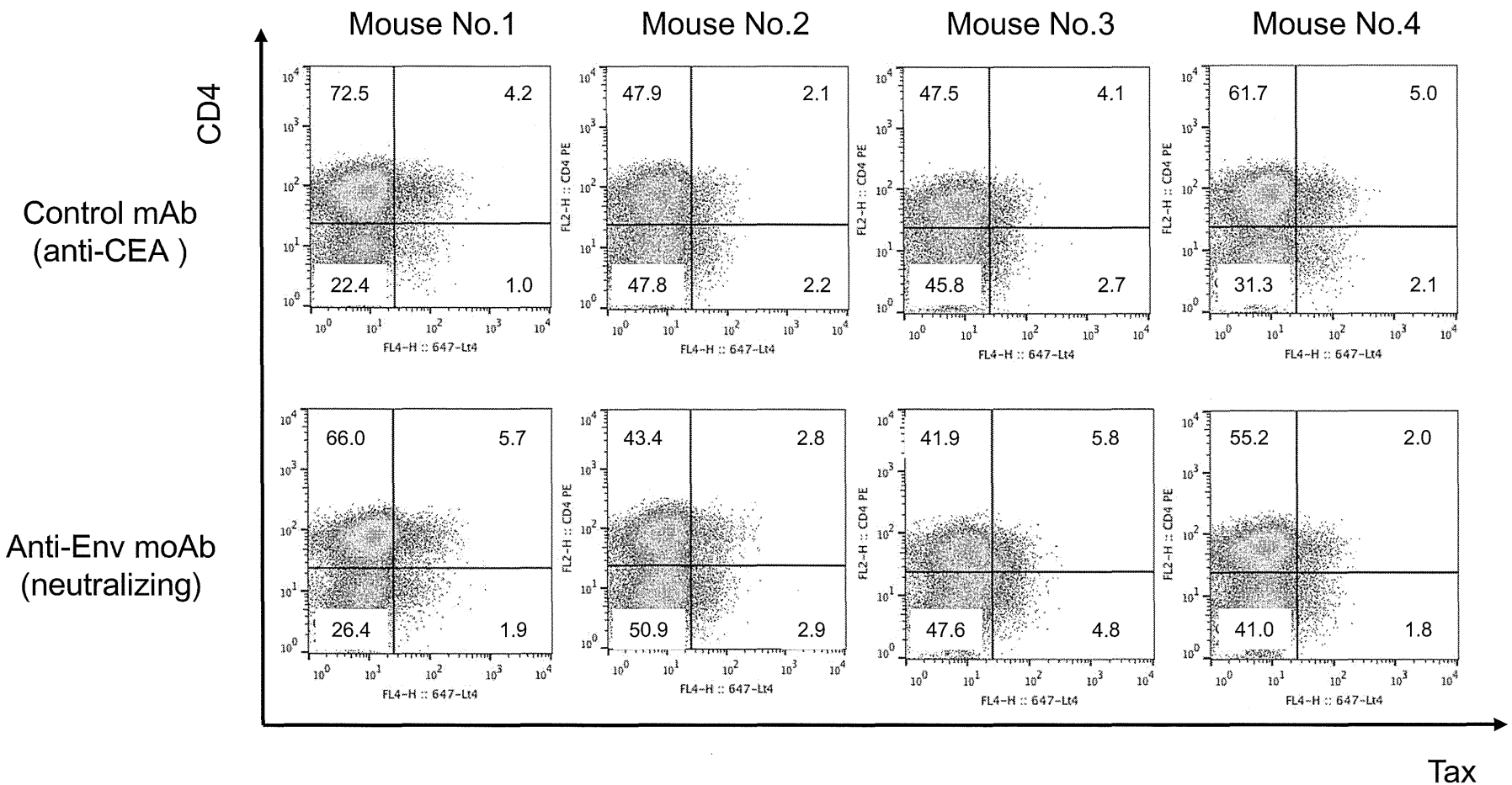
Supplemental Figure 1



Flow cytometric studies showed that the human lymphocytes recovered from mouse spleens express the amount of Tax protein after short-term (16 h) cultivation ex vivo, indicating that the neutralizing anti-Env Ab (clone LAT-27) injection once after PBMC transplantation did not block the in vivo transmission of HTLV-1.

Supplemental Figure 2