The Journal of Immunology



# Depletion of Dendritic Cells Enhances Susceptibility to Cell-Free Infection of Human T Cell Leukemia Virus Type 1 in CD11c-Diphtheria Toxin Receptor Transgenic Mice

Saifur Rahman, Sharrón L. Manuel, Zafar K. Khan, Brian Wigdahl, Edward Acheampong, Frederic Tangy and Pooja Jain

*J Immunol* 2010; 184:5553-5561; Prepublished online 9 April 2010;
doi: 10.4049/jimmunol.0903226
http://www.jimmunol.org/content/184/10/5553



*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

# Depletion of Dendritic Cells Enhances Susceptibility to Cell-Free Infection of Human T Cell Leukemia Virus Type 1 in CD11c-Diphtheria Toxin Receptor Transgenic Mice

**Saifur Rahman,[*,†] Sharrón L. Manuel,[*,†] Zafar K. Khan,[*,†] Brian Wigdahl,[†] Edward Acheampong,[*,†] Frederic Tangy,[‡] and Pooja Jain[*,†]**

**Human T cell leukemia virus type 1 (HTLV-1) is associated with two immunologically distinct diseases: HTLV-1–associated myelopathy/tropical spastic paraparesis and adult T cell leukemia. The genesis of these diseases is believed to be associated with the route (mucosa versus blood) and mode (cell-free versus cell-associated) of primary infection as well as the modulation of dendritic cell (DC) functions. To explore the role of DCs during early HTLV-1 infection in vivo, we used a chimeric HTLV-1 with a replaced envelope gene from Moloney murine leukemia virus to allow HTLV-1 to fuse with murine cells, which are generally not susceptible to infection with human retroviruses. We also used a CD11c-diphtheria toxin receptor transgenic mouse model system that permits conditional transient depletion of CD11c$^+$ DCs. We infected these transgenic mice with HTLV-1 using both cell-free and cell-associated infection routes in the absence and presence of DCs. The ablation of DCs led to an enhanced susceptibility to infection with cell-free but not cell-associated HTLV-1 in both CD4 and non-CD4 fractions, as measured by the proviral load. Infection with cell-free virus in the absence of DCs was also found to have increased levels of Tax mRNA in the non-CD4 fraction. Moreover, depletion of DCs significantly dampened the cellular immune response (IFN-$\gamma^+$CD8$^+$ T cells) against both cell-free and cell-associated virus. These results uniquely differentiate the involvement of DCs in early cell-free versus late cell-associated infection of HTLV-1 and highlight a significant aspect of viral immunopathogenesis related to the progression of adult T cell leukemia and HTLV-1–associated myelopathy/tropical spastic paraparesis after the initial infection.** *The Journal of Immunology*, **2010, 184: 5553–5561.**

Approximately 10–20 million people are infected with human T cell leukemia virus type 1 (HTLV-1) worldwide; endemic regions occur in Japan, the Caribbean, and parts of South America and Central Africa (1). A majority of HTLV-1–infected individuals remain asymptomatic carriers; only a small percentage (<5%) develops the disease (2, 3). HTLV-1 therefore represents a classic example of a persistent retroviral infection that has adapted to coexist with humans through the ages. Why certain infected individuals develop the disease whereas others remain as healthy carriers is not known. Etiological studies have linked HTLV-1 with many different clinical syndromes (4) and two predominant, immunologically distinct diseases: oncogenic adult T cell leukemia (ATL) (5) and the neuroinflammatory HTLV-1–associated myelopathy and tropical spastic paraparesis (HAM/TSP) (6, 7). The clinical outcome following HTLV-1 infection may be associated with the age as well as route of primary infection. It has been suggested that the risk of developing HAM/TSP is higher if HTLV-1 infection is acquired during adulthood especially through the sexual transmission (8). However, individuals infected early in life through breastfeeding are believed to be at a greater risk to develop ATL because the infected immature thymocytes have more chances to develop into malignant cells (9). The i.v. route of viral transmission has a predisposition to lead to the development of the neurologic disease associated with hyperimmune responses, as was shown in a cohort of HAM patients, most of whom received blood transfusions (10). On the contrary, rats orally inoculated with HTLV-1 developed persistent infection, immune unresponsiveness, and T cell lymphomas (11, 12), suggesting that mucosal exposure may lead to ATL.

HTLV-1 is an exogenous retrovirus that can replicate by either the infectious or the mitotic route, with the latter being the preferred mode of replication (13). Active expression of proviral DNA and the subsequent release of viral particles that infect other target cells lead to the infectious cycle of viral spread. Conversely, the mitotic cycle helps to increase the proviral load through the clonal expansion of such HTLV-1–infected cells by using host DNA polymerase instead of the error-prone viral reverse transcriptase (13–15). HTLV exists primarily as a cell-associated provirus and is transmitted preferentially through cell-to-cell contact by cellular cytoskeleton reorganization at the membrane interface (16), a mechanism that has been similarly demonstrated for HIV-1 (17, 18). The virus primarily targets CD4$^+$ T cells (19), but other secondary target cell types, such as CD8$^+$ T cells (20, 21), dendritic cells (DCs) (22), as well as those belonging to the CNS (23), such as astrocytes, microglia, and cells of the monocytic/macrophage lineage within the CNS, are also infected (24–27). The DCs are of particular significance with regard to HTLV-1 pathogenesis because the development of the hyperinflammatory condition HAM/TSP has been associated with the rapid maturation of DCs

*Drexel Institute for Biotechnology and Virology Research and †Department of Microbiology and Immunology, Center for Cancer Biology and Center for Molecular Virology and Neuroimmunology, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19127; and ‡Unité des Virus Lents, Unité de Recherche Associée, 1930 Centre Nationale de Recherche Scientifique, Institut Pasteur, Paris, France

Received for publication October 2, 2009. Accepted for publication March 5, 2010.

This work was supported by the United States Public Health Service and National Institutes of Health Grants R01 AI077414 (to P.J.) and R01 CA054559 (to B.W.).

Address correspondence and reprint requests to Dr. Pooja Jain, Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, 3805 Old Easton Road, Doylestown, PA 18902. E-mail address: pjain@drexelmed.edu

Abbreviations used in this paper: ATL, adult T cell leukemia; CM, control mouse; *Ct*, threshold cycle; DC, dendritic cell; DT, diphtheria toxin; DTR, diphtheria toxin receptor; HAM/TSP, HTLV-1–associated myelopathy/tropical spastic paraparesis; HTLV-1, human T cell leukemia virus type 1; M1, infected mouse 1; M2, infected mouse 2; M3, infected mouse 3; Mo-MLV, Moloney murine leukemia virus; NC, negative control; PC, positive control; w/o, without.



www.jimmunol.org/cgi/doi/10.4049/jimmunol.0903226

(28), whereas the immunosuppressive condition ATL involves a maturation defect in this cell population (29–31). HTLV-1 infection of DCs has been demonstrated in HAM/TSP patients (32) as well as in vitro (28, 33, 34). In addition, autologously infected DCs as well as those pulsed with inactivated HTLV-1 virions can lead to a strong proliferative response of both $CD4^+$ and $CD8^+$ T cells (35). We showed previously that DCs exhibit higher binding affinity to cell-free HTLV-1 than other susceptible cell types, including $CD4^+$ T cells (36), the primary target cell population in patients. Additionally, we and others have demonstrated that cell-free HTLV-1 productively infects DCs, which can in turn transfer the virus to $CD4^+$ T cells (33, 34, 37), suggesting that DCs play a critical role in primary infection and subsequent transmission. A unique feature of HTLV-1–associated neuroinflammatory disease is the intense proliferation of $CD8^+$ T cells, the majority of which are targeted against the viral transcription transactivating protein Tax (38). We showed previously that, following exposure to Tax, the murine DC cell line JAWS II as well as human monocyte-derived DCs undergo activation and maturation, exhibiting changes in mRNA levels of activation markers, surface phenotype, and secretion of cytokines/chemokines leading to allogenic and Ag (Tax)-specific immune responses (39–42). In addition, we showed that DCs can prime a functional Tax-specific cytotoxic T cell response in naive human PBLs in vitro and in the line HHD II HLA-A*0201 transgenic mice in vivo (34). With these compelling findings from the murine DC cell line JAWS II and Tax immunization results from the line HHD II mice, we addressed whether DCs play a role in determining the degree of infection engendered by cell-free and cell-associated routes using a realistic infection model system.

The inefficient fusion of murine cells and HTLV-1 has been overcome by a chimeric HTLV-1 wherein the envelope gene of HTLV-1 was replaced with that of the ecotropic Moloney murine leukemia virus (Mo-MLV) (43, 44). A similar approach was used to study HIV-1 pathogenesis in mice (45). We used C57BL/6-CD11c-diphtheria toxin receptor (DTR) transgenic mice, which express the high-affinity simian DTR coupled to the murine CD11c promoter, thereby allowing the selective depletion of the $CD11c^+$ DCs through the administration of diphtheria toxin (DT) (46). Because the chimeric HTLV-1 had not been tested in C57BL/6 mice, we first determined the susceptibility and infection of the chimeric virus on this background before studying DCs' involvement in two routes of infection: cell-free and cell-associated. Our studies show that depletion of DCs increases the susceptibility of CD11c-DTR transgenic mice to infection with cell-free virus in both CD4 and non-CD4 fractions. During cell-associated infection, no major difference was observed in the levels of infection in the absence or presence of DCs. However, a reduced proviral load was seen in the $CD4^+$ fraction in the DC-depleted group, suggesting that DCs may be important for the transmission of the virus to the $CD4^+$ population in a cell-associated manner. The $CD8^+$ T cells from the DC-depleted group showed a reduced ability to generate an IFN-γ response compared with those from the nondepleted group, thereby correlating with the infection results. These findings demonstrate that during the initial phase of HTLV-1 infection DCs may be critical to the control of cell-free virus; however, during cell-associated infection, they may facilitate *trans*-infection to T cells, as is known for other viruses.

## Materials and Methods

### *Mice*

Breeding pairs of B6.FVB-Tg Itgax-DTR/EGFP 57Lan/J (CD11c-DTR) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The CD11c-DTR mice express the transgene encoding for the simian DTR-GFP fusion protein, which is regulated under the control of the CD11c promoter (46). Administration of DT (Sigma-Aldrich, St. Louis, MO) resulted in the selective ablation of $CD11c^+$ cells. The genotypes of the pups were determined by PCR using DNA obtained from tail clippings using specific primer sequences (46). Age- and sex-matched mice between the ages of 6 and 10 wk were used. All of the animal experiments were performed as per institutional guidelines.

### *Production of cell-free virus*

The chimeric HTLV-1 plasmid (pCS-HTLV-chimΔR) encoding the HTLV-1 with the Mo-MLV envelope and the Tax-expressing plasmid (pCMV-env*ΔPvu*II) were obtained from Dr. Frederic Tangy (Institut Pasteur, Paris, France) (43, 44). The HEK-293T cells were cotransfected with 3 μg pCS-HTLV-chimΔR and 0.5 μg pCMV-env*ΔPvu*II per million cells by the calcium phosphate (Invitrogen, Carlsbad, CA) method. Five hours posttransfection, the medium was washed off and replaced with fresh medium. The successful production of virus was confirmed 36 h posttransfection, in both the supernatant and the cell lysate of transfected cells by the HTLV-1 Ag (p19) ELISA kit (ZeptoMetrix, Buffalo, NY). For the cell-associated infection, transfected

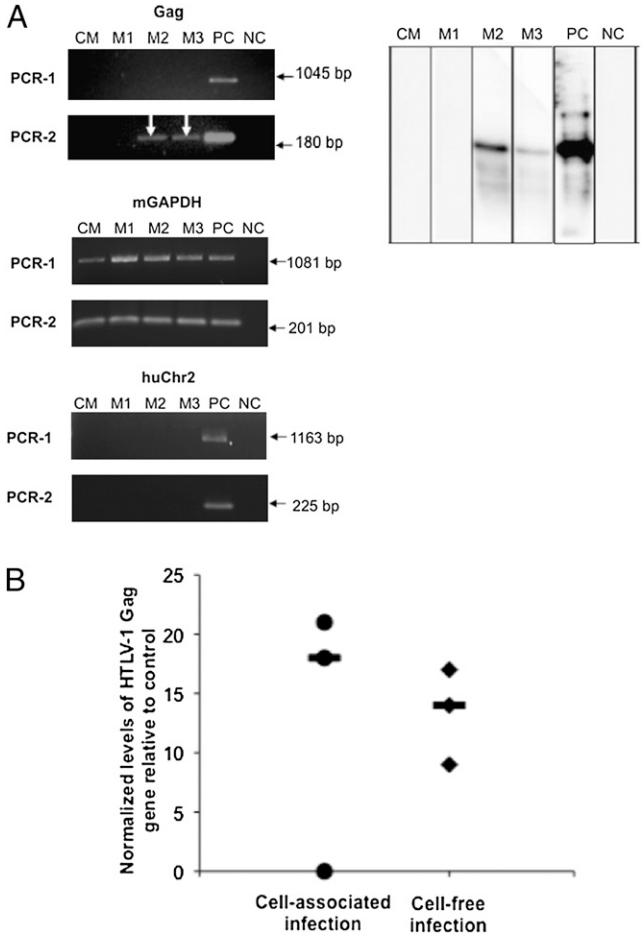


**FIGURE 1.** Qualitative detection of HTLV-1 infection on day 5 from splenocytic DNA of one control and three infected mice. *A*, Cell-associated HTLV-1 infection: nested PCR showing amplification of the Gag gene in PCR-2 from two of three infected mice. Demonstration of the presence of mouse GAPDH (positive control) and the absence of human chromosome 2 (negative control) in the DNA preparation from the mice splenocytes by nested PCR. Southern blot hybridization was performed to detect Gag PCR-1 product. *B*, Relative levels of proviral load (HTLV-1 Gag) in the two different infection groups measured by real-time PCR and normalized to the endogenous mouse GAPDH. The representative data are from three mice per group. The bar represents the median. Mouse GAPDH values serve as positive controls for all four samples tested, whereas human chromosome 2 was used as a negative control and could not be detected. The real-time PCR was independently repeated three times with each sample in duplicate. CM, control mouse; M1, infected mouse 1; M2, infected mouse 2; M3, infected mouse 3; NC, negative control; PC, positive control.

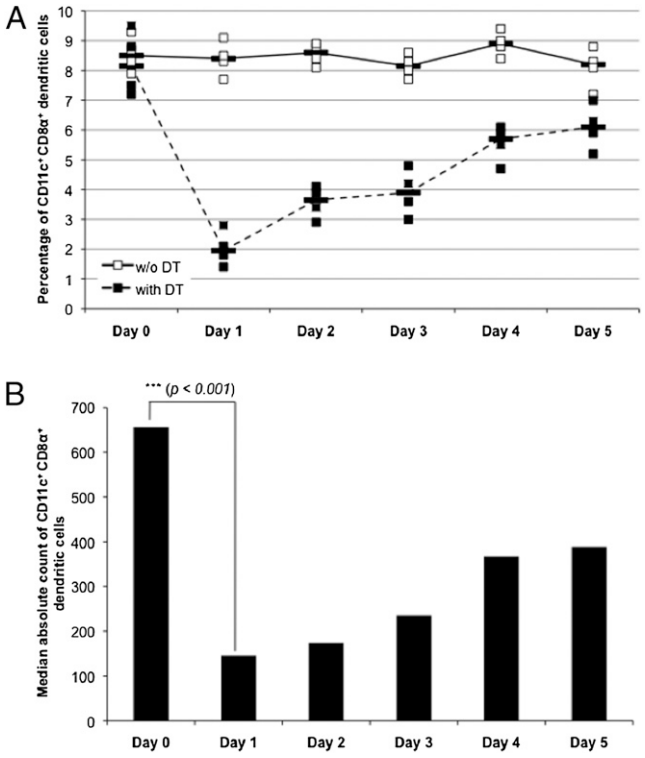


**FIGURE 2.** Kinetics of dendritic cells during depletion with DT from days 0–5. *A*, Percentage and median of $CD11c^+CD8\alpha^+$ DCs in DTR transgenic mice. *B*, Median absolute number of $CD11c^+CD8\alpha^+$ DCs in DTR transgenic mice. The representative data are from five mice per group per condition per time point. Statistical significance (***$p < 0.001$) between day 0 and day 1 determined by Student *t* test.

cells were pooled and injected into the mice. To obtain a sample of concentrated cell-free virus, the collected supernatant was first passed through a 0.45-μm filter (Millipore, Bedford, MA) and then overlaid on a 20% (w/v) sucrose cushion in polyallomer tubes (Beckman Coulter, Fullerton, CA). The virus particles were concentrated from the filtered supernatant by ultracentrifugation for 2 h at 25,000 rpm on the Beckman Optima CE-80K (SW28.1; Beckman Coulter). The virus pellet was resuspended in 1:100 volume of PBS and quantified before use by HTLV-1 p19 ELISA (ZeptoMetrix).

### *Administration of DT and virus*

The mice were given (i.p.) only PBS as the control or a uniform dose of 100 ng DT (~5 ng DT per gram of body weight) DT (Sigma-Aldrich) resuspended in water. Approximately 20 h later, the virus was administered to the mice in either cell-associated or cell-free form. For cell-associated viral infection, each mouse was injected (i.p.) with $10^7$ transfected HEK-293T cells as described previously (44). For cell-free viral infection, $0.5 \times 10^6$ pg p19 virus was injected (i.p.) in each mouse. The mice were sacrificed on day 5 postviral challenge, and their spleens were harvested for further analyses.

### *$CD4^+$ T cell separation from splenocytes*

The spleens of the sacrificed mice were harvested in RPMI 1640 media (Mediatech, Manassas, VA), macerated, and passed through a 0.45-μm sterile nylon filter (BD Biosciences, San Jose, CA). The splenocytes were then subjected to $CD4^+$ separation using the EasySep mouse $CD4^+$ T cell enrichment kit (STEMCELL Technologies, Vancouver, BC, Canada). DNA was subsequently isolated from the $CD4^+$ T cell fraction using the SV Wizard Genomic DNA Purification System kit (Promega, Madison, WI), whereas both DNA (Promega) and RNA (RNeasy Mini Kit; Qiagen, Valencia, CA) were isolated from the non-CD4 fraction. From the isolated RNA, 2 μg was then reverse-transcribed into cDNA using the Omniscript RT kit (Qiagen). The isolated DNA was used to determine the proviral load in both CD4 and non-CD4 fractions, whereas the RNA isolated only from the non-CD4 fraction was used to determine the Tax mRNA load.

### *Real-time PCR*

The DNA from both CD4 and non-CD4 fractions was subjected to real-time PCR analysis to detect the HTLV-1 Gag gene; similarly, the cDNA was used to detect Tax mRNA. Real-time PCR was performed on an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). The standard real-time PCR reaction using SYBR Green I consisted of 15 μl SYBR Green PCR Master Mix (Applied Biosystems), 10 μM each of forward and reverse primers (Integrated DNA Technologies, Coralville, IA), and 10 μl of DNA in a total volume of 30 μl. The thermal cycling conditions comprised an initial activation step at 95°C for 10 min followed by 40 cycles including denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min. The following primers were used to detect HTLV-1 Gag (forward, 5′-CCTTACCACGCCTTCGTAGA-3′; reverse, 5′-ACAAGCCCGCAACATATCTC-3′), mouse GAPDH (forward, 5′-CCTTAAACAGGCCCACTTGA-3′; reverse, 5′-CCTTCCACAATGCCAAAGTT-3′), and human chromosome 2 (forward, 5′-CTGCCATTGACAGCAACACT-3′; reverse, 5′-TTGGGCACTGACAAGTGAAG-3′), yielding 180, 201, and 225 bp products, respectively. The following primers were used for HTLV-1–spliced Tax mRNA (47) (RPX3, 5′-ATCCCGTGGAGACTCCTCAA-3′; RPX-4+3, 5′-CCAAACACGTAGACTGGGTATCC-3′) and for mouse GAPDH coding sequence (forward, 5′-TGTGTCCGTCGTGGATCTGA-3′; reverse, 5′-CCTGCTTCACCACCTTCTTGA-3′). By use of the $2^{-\Delta\Delta CT}$ method (48), the levels of HTLV-1 Gag gene and Tax mRNA were quantified relative to the control group and normalized to the endogenous housekeeping GAPDH. Experiments were performed in duplicate for each data point. Dissociation or melting curve analysis was performed to ensure the presence of a single peak at the correct melting temperature.

### *Nested PCR*

For nested PCR, two primer sets (Integrated DNA Technologies) were used to detect the HTLV-1 Gag gene. The first primer set (forward, 5′-TAGCCAGCCTACTCCCAAAA-3′; reverse, 5′-GCGGGGAGGTCTAATAGGAG-3′) amplified a 1045-bp product of the Gag gene, whereas the second set (forward, 5′-CCTTACCACGCCTTCGTAGA-3′; reverse, 5′-ACAAGCCCGCAACATATCTC-3′) amplified a 180-bp product within the first PCR-amplified product. Two control PCR reaction sets were also performed, the first using mouse GAPDH as a positive control to demonstrate that the isolated DNA is indeed from mouse splenocytes and the second using human chromosome 2 as a negative control to abrogate the question of DNA contamination from the transfected HEK-293T cells. The first set for mouse GAPDH (forward, 5′-ATGAGTGATGGGGGTGATGT-3′; reverse, 5′-CCTTCCACAATGCCAAAGTT-3′) yielded a 1081-bp product, whereas the second set (forward, 5′CCTTAAACAGGCCCACTTGA-3′; reverse, 5′-CCTTCCACAATGCCAAAGTT-3′) gave a 201-bp product. For human chromosome 2, the first primer set (forward, 5′-TTCTGGAGGTCAGCCTGTCT-3′; reverse, 5′-TCAGTGTGACCAGT

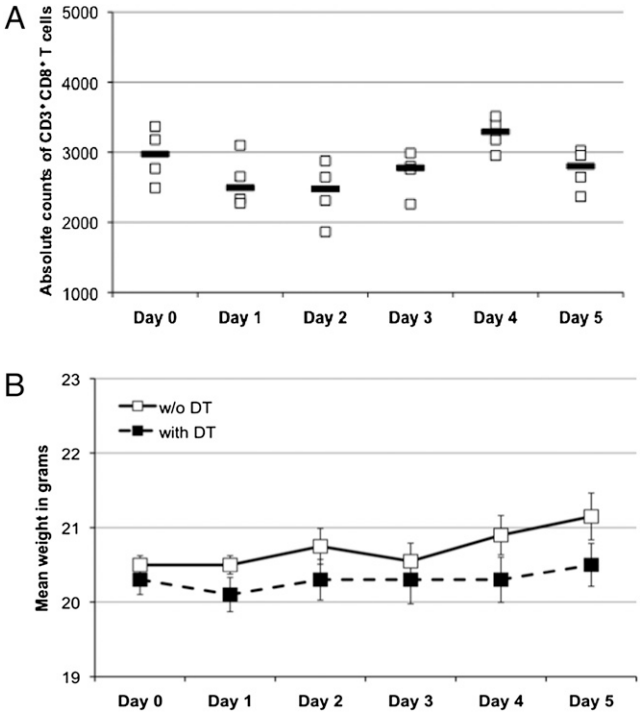


**FIGURE 3.** Kinetics of $CD8^+$ T cells and weight of mice from days 0–5. *A*, Total number and median of $CD3^+CD8\alpha^+$ T cells in DTR transgenic mice. *B*, Mean weights of control (PBS-treated) and experimental (DT-treated) mice. The representative data are from five mice per group per time point.

GGGGTA-3′) yielded a 1163-bp product, and the second set (forward, 5′-CTGCCATTGACAGCAACACT-3′; reverse, 5′-TTGGGCACTGACAA GTGAAG-3′) resulted in a 225-bp product. For the first PCR, 50 ng DNA was used as template and from which 3 μl was added to the second PCR as template. Both rounds of PCR were performed for 35 cycles (94°C for 30 s, 50°C for 30 s, and 72°C for 1 min) on the Mastercycler (Eppendorf, Westbury, NY) using the PCR SuperMix High Fidelity (Invitrogen).

### *Southern blot hybridization*

The Gag PCR-1 samples from nested PCR were used with the amplified positive control (1045 bp) as the probe that was end-labeled with α-[$^{32}$P] dCTP (Perkin Elmer, Waltham, MA) using the Amersham Rediprime II random prime labeling system kit as per the manufacturer's instructions (GE Healthcare, Piscataway, NJ). The membrane with the labeled probe was left for 24 h on the phosphor imager screen, which was then scanned

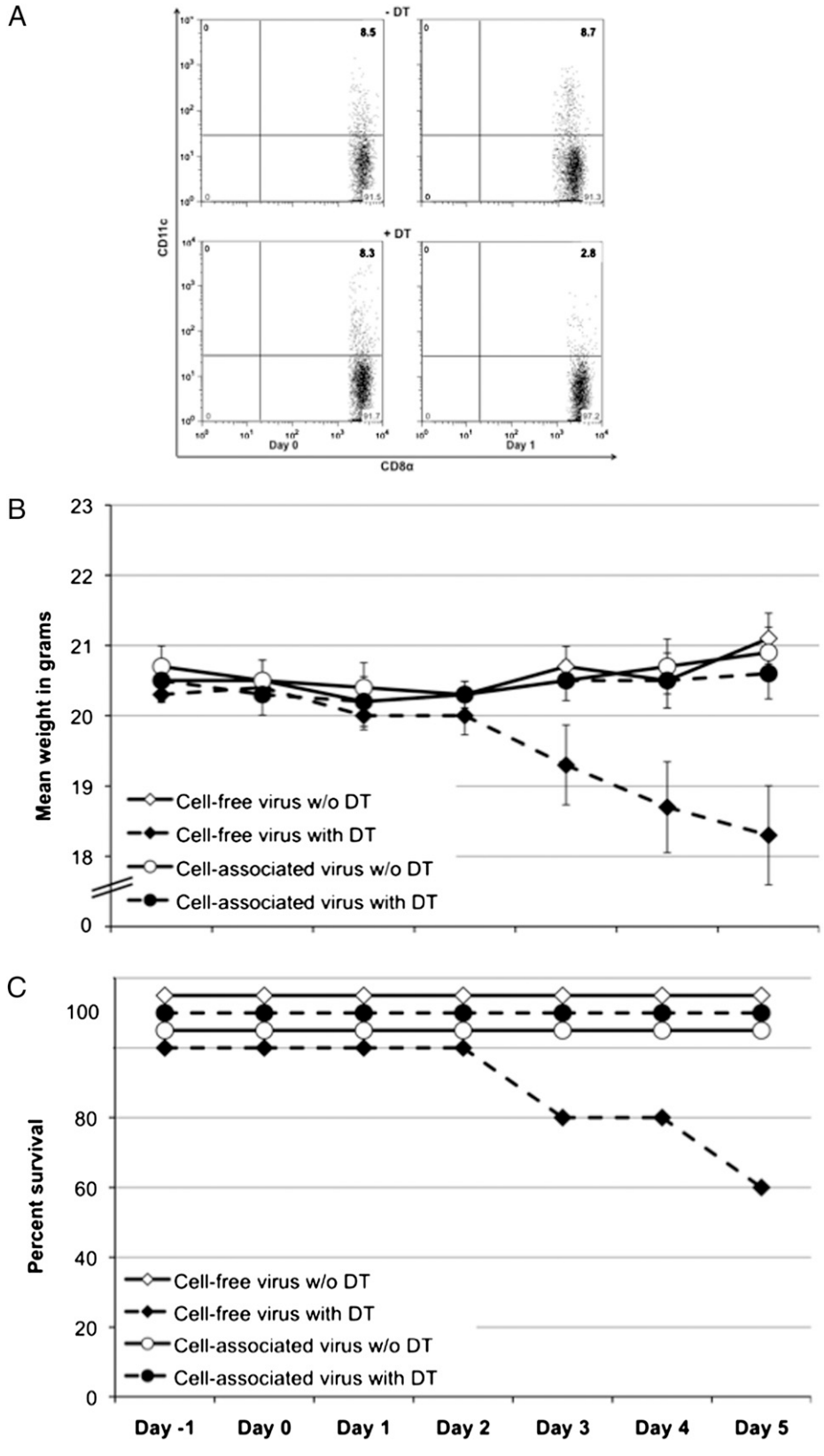


**FIGURE 4.** Weight chart and percentage survival rate of DTR mice infected with either cell-free or cell-associated virus in the absence or presence of DCs. On day −1, the control mice were given PBS (white circle), whereas the experimental mice were given DT (black circle). The next day (day 0), both groups were given the virus in either cell-free (diamonds) or cell-associated (circles) form. *A*, Confirmation of depletion of DCs in the DT-treated group on day 0. *B*, Mean weight chart for the four different groups. *C*, Percentage survival of the different groups. The representative data are from five mice per group per condition per time point with standard deviations.

on the STORM 820 scanner (Amersham Biosciences, Piscataway, NJ) and analyzed using the ImageQuant software (version 2003).

### *In vitro stimulation experiment*

After CD4 separation, cells from the non-CD4 fraction were set up for an in vitro stimulation experiment to examine IFN-γ production. With a 24-well plate, $1 \times 10^6$ cells were either left untreated as controls or pulsed with 5 μg/ml concavalin A (Sigma-Aldrich) or 100 pg p19 HTLV-1 virus. The cells were then collected, stained with anti-mouse mAbs, and analyzed by flow cytometry.

### *Abs for flow cytometric analyses*

To detect mouse T cells and DCs, the cells were stained using fluorochrome-labeled anti-mouse mAb for mouse surface markers CD3, CD4, CD8α, and CD11c (eBioscience, San Diego, CA). For in vitro analyses, the cells were treated with brefeldin A (BD GolgiPlug; BD Biosciences) for 5 h prior to initiating staining of cells. After surface staining, the cells were permeabilized using a BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit with BD GolgiPlug (BD Biosciences) and then stained intracellularly for mouse IFN-γ (eBioscience). The stained cells were analyzed by flow cytometry on the FACSCalibur (BD Biosciences), and the data were analyzed using FlowJo software (Tree Star, Ashland, OR).

## Results

### *C57BL/6 mice are susceptible to infection with the chimeric HTLV-1 virus*

The infectivity of the HTLV-1 chimeric virus was previously demonstrated in various mouse strains (BALB/c, C3H/He, 129Sv, and 129Sv IFNAR$^{-/-}$) but not in the C57BL/6 strain (44). Because the CD11c-DTR transgenic mice used in our studies were on the C57BL/6 background, we decided to test whether this mouse strain is susceptible to infection with both cell-associated and cell-free chimeric HTLV-1. For the cell-associated infection, we followed a previously described approach (44). Briefly, the wild-type C57BL/6 mice were injected (i.p.) with $10^7$ transfected HEK-293T cells. The mice were sacrificed on day 5, their spleens were harvested, and DNA was extracted from the isolated splenocytes. We detected proviral Gag DNA using three independent qualitative techniques—nested PCR and Southern blot hybridization (Fig. 1*A*) as well as real-time PCR (Fig. 1*B*). Of the three mice tested for cell-associated infection, two yielded positive results by all three techniques (Fig. 1). Because transfected human HEK-293T cells were injected into these mice, we confirmed that the extracted DNA was indeed coming from mouse splenocytes and not from the injected human cells by amplifying for mouse GAPDH as the positive control and for human chromosome 2 as the negative control (Fig. 1*A*). To test for infection by cell-free virus, each mouse was given $0.5 \times 10^6$ pg HTLV-1 p19 virus (i.p.) and sacrificed on day 5, and the isolated splenocytic DNA was analyzed by real-time PCR for HTLV-1 Gag. All three mice tested positive for HTLV-1 Gag (Fig. 1*B*). These results demonstrated that the C57BL/6 strain was infected by both cell-associated and cell-free chimeric HTLV-1.

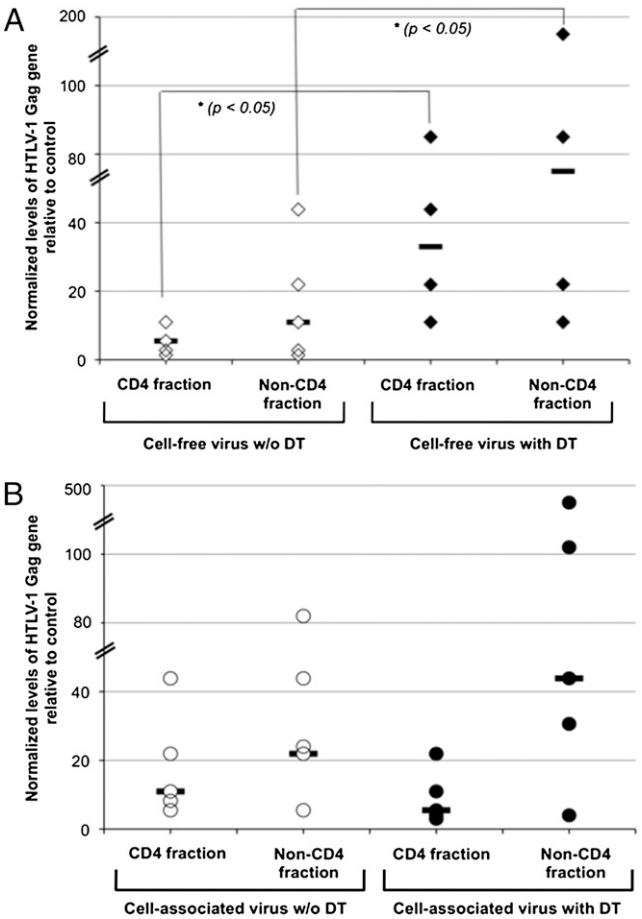


**FIGURE 5.** Relative levels of proviral load in the four different infected mice groups measured by HTLV-1 Gag amplification and normalized to the endogenous mouse GAPDH. *A*, *B*, Cell-free (*A*) and cell-associated (*B*) viral infection in the absence and presence of DCs. The representative data are from five mice per group. The bar represents the median. Statistical significance ($*p < 0.05$) determined by Student *t* test.

### *Kinetics of DC depletion in CD11c-DTR transgenic mice*

After demonstrating infection with chimeric HTLV-1 virus in C57BL/6 mice, we proceeded to determine the kinetics of DC depletion in our hand. On the basis of results from the literature, the mice were given a uniform dose of 100 ng DT (i.p.) and sacrificed on day 5. Flow cytometric analyses demonstrated a significant drop in the percentage of CD11c$^+$CD8α$^+$ conventional DCs starting on day 1, with gradual repopulation of depleted DCs from day 3 to day 5 (Fig. 2*A*). We also examined the absolute number of CD11c$^+$CD8α$^+$ cells and observed that the drop in DC numbers was very significant ($***p < 0.001$) after the administration of DT (Fig. 2*B*). Some studies have suggested that CD11c may be expressed on activated CD8$^+$ T cells (46, 49, 50); therefore, we also examined the absolute numbers of CD3$^+$CD8$^+$ T cells but observed no significant changes (Fig. 3*A*). To assess for the toxicity of DT, we compared mean body weight of mice given DT with that of control mice but observed no significant differences (Fig. 3*B*).

### *Mice are susceptible to cell-free virus in the absence of DCs*

After having demonstrated that the C57BL/6 strain can be infected with the chimeric HTLV-1 and standardizing the depletion of murine DCs in our laboratory settings, we then compared the two routes of infection in the absence and presence of DCs. Among the four groups tested ($n = 5$), two groups were administered DT and subsequently given either cell-free or cell-associated virus. The depletion of DCs was confirmed in all of the DT-treated animals as shown by the representative data in Fig. 4*A*. Overall, the group that had been depleted of DCs and given cell-free virus showed the most striking change. This group not only showed weight loss (Fig. 4*B*) but mortality as well (Fig. 4*C*); however, the cause of animal death is not very clear at this point.

We sacrificed these mice on day 5 and harvested their spleens. Interestingly, one mouse from the DC-depleted and cell-free virus group died on the last day (Fig. 4*C*) after having its weight taken, the spleen from which was immediately isolated and processed. Instead of examining the total splenocytic population, which is composed of different cell types, we separated the splenocytes into two fractions, CD4 and non-CD4, by enriching for the CD4$^+$ T cells from the total splenocytes. Because HTLV-1 is CD4-tropic and the tropism of the chimeric virus with the replaced Mo-MLV

is still considered to be CD4-tropic (43, 44), we determined the proviral load in both fractions and the Tax mRNA load in only the non-CD4 fraction. Using real-time PCR and the $2^{-\Delta\Delta CT}$ method (48), we compared the relative levels of the HTLV-1 Gag gene in both fractions of the four experimental groups to the levels in the controls. We observed a marked significant ($*p < 0.05$) increase in the relative levels of HTLV-1 Gag in both CD4 and non-CD4 fractions of mice depleted of DCs and given cell-free virus compared with those not depleted of DCs (Fig. 5*A*). This result clearly demonstrates that the susceptibility to HTLV-1 infection of the $CD4^+$ T cells, which are the primary target of the virus, as well as other target cells in the non-CD4 fraction increases in the absence of the sentinel DCs. Although the proviral load was slightly higher in the non-CD4 fraction compared with that in the CD4 fraction, the observed difference was not statistically significant. This slight increase could be due to the fact that the non-CD4 fraction contains many different cell types (i.e., $CD8^+$ T cells, B cells, macrophages, and NK cells), all of which can be infected by HTLV-1 to varying degrees. In fact, previous work with the chimeric HTLV-1 virus also demonstrated greater infection in the $CD4^-/CD8^-$ fraction compared with that in the $CD4^+$ fraction (44).

However, when we compared the relative levels of infection in the case of cell-associated viral infection, we observed no significant differences ($p < 0.5$) in either the CD4 or the non-CD4 fraction in the absence or presence of DCs (Fig. 5*B*). These data indicate that, in the case of cell-associated infection, the HTLV-1 virus is rarely exposed to the monitoring DCs and that the absence or presence of DCs therefore does not make much difference in determining the level of infection, unlike the situation seen with the cell-free virus. It is well known that HTLV-1 prefers cell-to-cell–mediated transmission and is rarely found as free budding virus (16). Therefore, unlike cell-free virus, it is not easily available to the DCs. When we compared the individual changes in weight and proviral load in both CD4 and non-CD4 fractions for each mouse in all four groups, we observed a significant weight loss in the mice that were depleted of DCs and given cell-free virus (Table I). The same group also demonstrated a much higher proviral load in both CD4 and non-CD4 fractions compared with those in the other groups (Table I). Overall, these data suggest that cell-free virus can induce an increased level of infection with greater proviral load in the absence of DCs. These results confirm the critical role of DCs in controlling cell-free virus.

### *Increased Tax mRNA load in mice depleted of DCs*

To determine whether both the cell-free and the cell-associated viruses were able to replicate in mice, we looked at the expression of the viral transcriptional transactivating protein Tax by determining the mRNA levels from the non-CD4 fraction. After reverse transcription of the isolated RNA into cDNA, we performed real-time PCR using primers for spliced Tax mRNA (47). Using the $2^{-\Delta\Delta CT}$ method (48), we compared the levels of HTLV-1 Tax mRNA in the non-CD4 fraction of the four experimental groups relative to that in the control. We observed a significant increase ($*p < 0.05$) in the relative levels of Tax mRNA in mice depleted of DCs and given cell-free virus (Fig. 6), which correlates with the increased levels of HTLV-1 Gag DNA observed in the same group (Fig. 5*A*). However, no change was observed in the cell-associated infection group in either the absence or the presence of DCs (Fig. 6). These results suggest that in the absence of surveillance by DCs, cell-free virus can easily infect the primary target $CD4^+$ T cells as well as other cells (as seen in the non-CD4 fraction, Fig. 5*A*) and then begin to transcribe viral (Tax) mRNA at an increased rate, as seen in the non-CD4 fraction (Fig. 6), leading to a heightened state of infection.

### *Dampened cellular immune response in the absence of DCs*

The role of DCs, compared with the roles of other APCs, such as macrophages and B cells, in controlling viral infection has been demonstrated using numerous murine infection models (46, 51,

Table I. *Comparative analysis of change in weight and proviral load in both CD4 and non-CD4 fractions of four groups of mice*

| Group | Mouse | Weight (in Grams)[a] | | | Proviral Copies per 100 Cells[b] | |
|---|---|---|---|---|---|---|
| | | Day −1 | Day 5 | Change in Weight | CD4 Fraction | Non-CD4 Fraction |
| Cell-free virus w/o DT | M1 | 20.5 | 20.8 | 0.3 | 67 | 65 |
| | M2 | 20.4 | 20.7 | 0.3 | 63 | 63 |
| | M3 | 20.6 | 21.0 | 0.4 | 65 | 69 |
| | M4 | 20.8 | 21.2 | 0.4 | 67 | 71 |
| | M5 | 20.2 | 20.4 | 0.2 | 69 | 74 |
| | Median | 20.5 | 20.8 | 0.3 | 67 | 69 |
| Cell-free virus with DT | M1 | 20.3 | 18.5 | -1.8 | 69 | 79 |
| | M2 | 20.7 | 20.1 | -0.6 | 71 | 76 |
| | M3 | 20.5 | 18.3 | -2.2 | 74 | 69 |
| | M4 | 19.8 | 16.4 | -3.4 | 76 | 71 |
| | M5 | 20.2 | died | – | – | – |
| | Median | 20.3 | 18.4 | -1.9 | 72 | 74 |
| Cell-associated virus w/o DT | M1 | 20.7 | 20.9 | 0.2 | 74 | 71 |
| | M2 | 20.5 | 20.7 | 0.2 | 68 | 74 |
| | M3 | 20.3 | 20.5 | 0.2 | 67 | 76 |
| | M4 | 20.9 | 21.1 | 0.2 | 71 | 71 |
| | M5 | 21.0 | 21.2 | 0.2 | 69 | 67 |
| | Median | 20.7 | 20.9 | 0.2 | 69 | 71 |
| Cell-associated virus with DT | M1 | 20.1 | 20.4 | 0.3 | 65 | 68 |
| | M2 | 20.4 | 20.6 | 0.2 | 67 | 85 |
| | M3 | 20.7 | 20.9 | 0.2 | 69 | 72 |
| | M4 | 20.3 | 20.6 | 0.3 | 71 | 78 |
| | M5 | 20.8 | 21.0 | 0.2 | 67 | 67 |
| | Median | 20.4 | 20.6 | 0.2 | 67 | 72 |

[a]Change in weight was determined between day −1 and day 5.

[b]Proviral load in both CD4 and non-CD4 fractions was calculated by taking the ratio of the *Ct* value for Gag obtained from 1 μg TARL-2 [which is known to have a single copy of HTLV-1 provirus (62)] DNA and 1-μg sample DNA, which yields the number of copies per cell. This value was then multiplied by 100 to obtain the number of copies per 100 cells.

*Ct*, threshold cycle; w/o, without.

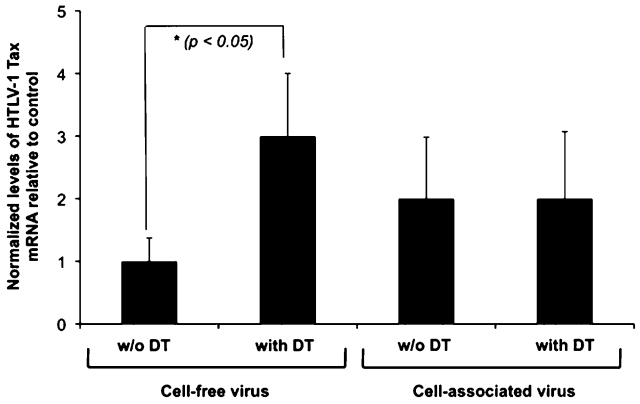


**FIGURE 6.** Relative mean levels of Tax mRNA load in the non-CD4 fraction of the four different groups of infected mice measured by Tax amplification and normalized to the endogenous mouse GAPDH mRNA. The representative data are from five mice per group. Error bars represent SE of mean. Statistical significance ($*p < 0.05$) between cell-free virus without and with DT, determined by Student *t* test.

52). After observing significantly increased proviral and Tax mRNA loads in mice depleted of DCs and given cell-free virus (Figs. 5, 6), we compared the quality of the cellular immune response in these mice to that in the group not depleted of DCs. We analyzed in vitro the IFN-γ response of the $CD8^+$ T cells following challenge with virus. The cells from the non-CD4 fraction were pulsed with either media as a control or virus and then 5 h later treated with brefeldin A to block IFN-γ secretion. Thereafter, the cells were surface-stained for CD3 and CD8 and intracellularly for IFN-γ. We looked at the absolute numbers (Fig. 7) and percentages (data not shown) of IFN-$\gamma^+CD8^+$ T cells from both groups. With cell-free infection, we observed a very significant reduction ($**p < 0.005$) in the ability of the $CD8^+$ T cells from mice depleted of DCs to produce IFN-γ when challenged with virus compared with that of those from mice that were not depleted of DCs (Fig. 7*A*). With the cell-associated infection group (Fig. 7*B*), we observed a similarly significant trend ($*p < 0.05$); however, the response was not as pronounced as that observed with cell-free infection. The reduced ability of the $CD8^+$ T cells to produce IFN-γ in response to viral challenge in mice depleted of DCs in both cell-free and cell-associated infection groups demonstrates the critical role of this unique immune cell type in warding off and controlling the viral threat.

## Discussion

Under natural circumstances, the HTLV-1 virus can enter the body either through the mucosal surface or the peripheral blood. With mucosal exposure, as hypothesized for the genesis of ATL (11, 12), the resident DCs can capture the virus, process it, and then present the Ag to the $CD8^+$ T cells for targeted lysis of infected cells. However, during the course of infection in ATL, the DCs exhibit defective maturation, thereby leading to a subdued cellular immune response (29). However, peripheral blood exposure, as hypothesized for the genesis of HAM/TSP (10), can lead to the direct infection of target $CD4^+$ T cells or can be picked up by the blood DCs. The DCs from such patients exhibited a matured phenotype and induced rapid proliferation of lymphocytes (28). Moreover, the depletion of DCs from such patients' PBMCs abrogated the spontaneous proliferation of lymphocytes, whereas proliferation could be restored by supplementing with DCs but not with either of the other APCs—B cells or macrophages. In addition, such spontaneous proliferation of lymphocytes can be blocked by mAbs to

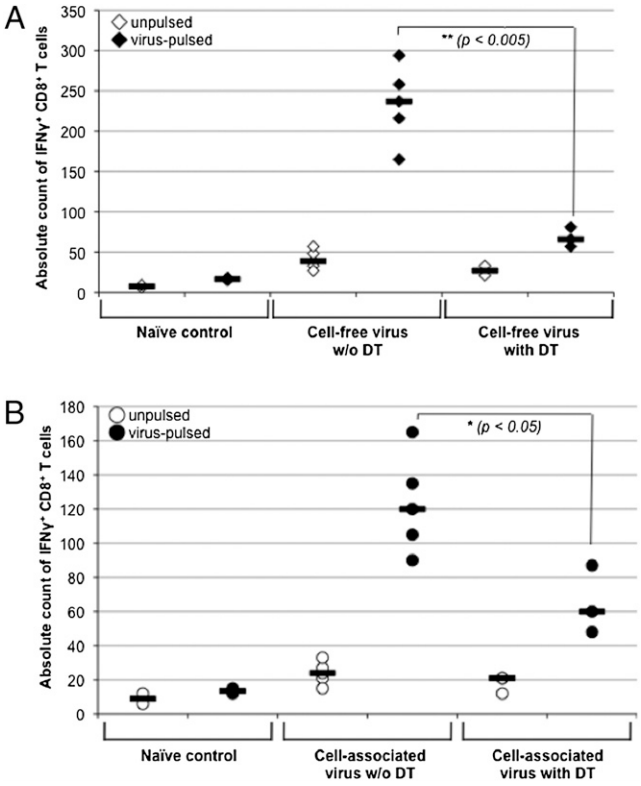


**FIGURE 7.** Cellular immune response for both infection groups in the absence and presence of DCs. Graphs represent the absolute numbers of IFN-$\gamma^+CD8^+$ T cells from DC-depleted and nondepleted mice when pulsed in vitro with media or virus. Immune response represented by the cell-free (*A*) and cell-associated (*B*) infections. The bar represents the median. Error bars represent SD. Statistical significance for both groups determined by Student *t* test.

MHC class II, CD86, and CD58, thus demonstrating a DC-dependent mechanism (32). As a result of two contrasting end-disease states with starkly opposite differences in DC maturation and activation, the role of DCs in HTLV-1 immunopathogenesis warrants further attention. Moreover, it has been suggested from some DC studies in HIV-1 and HTLV-1 that the infection of DCs can lead to the development of T cell abnormalities (53). For these reasons, we believe that, depending on the route of viral entry (mucosal versus peripheral blood) and mode of transmission (cell-free versus cell-associated), the virus can modulate the functions of DCs and thereby determine the quality of the immune response generated and possibly that of the end-disease state. As part of this hypothesis, we explored the role of DCs during cell-free and cell-associated early HTLV-1 infection in a mouse model system.

The complexity of the cytotoxic T cell response in HTLV-1 infection contributes to the lack of clarity regarding the disease pathogenesis that follows. To understand HTLV-1 disease, one must characterize the stages in early infection following the initial viral insult. Therefore, the interactions of DCs and T cells are important in understanding HTLV-1 infection. We showed previously that DCs exhibit a greater degree of binding to HTLV-1 virions compared with that of other cell types examined, including T cells, which are the main target of HTLV-1, and are highly susceptible to cell-free infection (36). Furthermore, DCs are heavily concentrated on mucosal surfaces, which are easily breeched by viruses and other pathogens. In addition, we and others have demonstrated the ability of DCs to transmit HTLV-1 to $CD4^+$ T cells (33, 37). Thus, DCs appear to be key in the early immune process. However, no suitable model exists to study the early stages of immune interactions in HTLV-1 infection. Therefore, we used the CD11c-DTR transgenic

mice as a tool to study the priming capacity of DCs in HTLV-1 infection in vivo.

An animal model allows us to answer many questions that could not be easily answered from studies in humans because of scarcity of patient samples and ethical constraints. The absence of a reliable animal model for HTLV-1 has long been a bottleneck and a debated issue in the field. Although many researchers have tried to use the mouse as a model system for HTLV-1 disease studies, they have been hampered by the poor infectivity of murine cells in response to infections with human retroviruses. Most of these studies involved i.p. inoculation of the HTLV-1 Tax-producing cell line MT2 into various strains of mice (54–56). The goal for most of these studies was to develop a mouse system for either of the HTLV-1–associated diseases: ATL or HAM/TSP. However, compared with the response of human cells, the main barrier to infection of murine cells with HTLV-1 is its inefficient fusion (57–60). Recently, a chimeric HTLV-1 was developed in which the HTLV-1 envelope gene was replaced by that of the ecotropic Mo-MLV, thereby facilitating fusion of murine cells with the chimeric virus containing the HTLV-1 genome (43, 44). This chimeric virus was infectious for a number of mouse strains tested (44). To understand the role of a particular cell type in vivo, a system in which hypotheses can be addressed and results compared in both the absence and the presence of the cell type in question offers a perfect model system. The CD11c-DTR transgenic mice are a useful tool for exploring the role of DCs in mice because they allow the selective transient depletion of DCs through the administration of DT (61). This model system demonstrates the critical role of DCs in the generation of a potent antiviral cellular immune response through the priming of naive $CD8^+$ T cells against both exogenous (46) and endogenous (51) cell-associated viral Ags. Using this chimeric virus, we compared cell-free and cell-associated HTLV-1 infections in both the absence and the presence of DCs using CD11c-DTR transgenic mice to better understand the role of DCs during HTLV-1 immunopathogenesis. This study is the first of its kind using mouse as a model system to explore the role of DCs in HTLV-1 immunopathogenesis.

It is difficult to have an exact comparison of the amount of virus used for the two routes of infection, especially postinoculation given the fact that the fates of cell-associated versus cell-free viruses could be different. Therefore, to equate the amount of administered virus via the two routes, we used HTLV-1 Gag (p19) ELISA units as the quantitative measure. The amount of virus collected from $10^7$ transfected HEK-293T cells has been generally found to be in the range of $0.3–0.4 \times 10^6$ pg p19; therefore, we used a slightly higher amount of virus ($0.5 \times 10^6$ pg p19) in the cell-free infection to compensate for the amount of virus lost during inoculation or those taken up by the local resident cell population in the peritoneum. As evident from the quantitative PCR data given in Table I, the number of proviral copies is fairly similar in both CD4 and non-CD4 fractions between cell-free and cell-associated infections in the absence of DT (control condition). Nevertheless, the main goal of this study was not to compare the efficiencies of cell-free versus cell-associated infections because the latter are already proven to be the preferred mode of HTLV-1 transmission. The primary purpose for these investigations was to investigate the role of DCs during HTLV-1 infection, transmission, or both using an in vivo system, as it has not been studied before. Previous in vitro data (33, 37) suggested that DCs are able to bind, internalize, and transfer HTLV-1 to T cells, and the current in vivo data indeed reveal a critical involvement of DCs in HTLV-1 pathogenesis, especially during early infection.

The present study clearly demonstrates that in the absence of DCs the cell-free HTLV-1 can lead to a greater degree of infection in contrast to cell-associated HTLV-1, as seen by weight loss and mortality of mice as well as increased proviral and Tax mRNA loads. These results are quite intriguing, highlighting the importance of DCs during the initial phase of HTLV-1 infection and in controlling viral spread. The ablation of this professional APC leads to a significantly diminished cellular ($CD8^+IFN\text{-}\gamma^+$) immune response (Fig. 7*A*), thereby allowing for greater proviral load as seen in both CD4 and non-CD4 fractions (Fig. 5*A*) as well as in an increased rate of viral transcription in the non-CD4 fraction (Fig. 6). Although no major difference was observed during cell-associated infection, the slightly reduced proviral load seen in the CD4 fraction of the DC-depleted mice suggests that DCs can help to transmit HTLV-1 to the $CD4^+$ T cells. As demonstrated in many previous studies, the DCs play a crucial role in the generation of a successful immune response, and its ablation severely abrogates the ability of the mice to respond to the viral challenge. Overall, these results offer a clear and real representation of the in vivo dynamics of DCs, particularly during the early phase of HTLV-1 infection. Parallels can be drawn from these results to help shed light on our understanding of the role of DCs in humans during early HTLV-1 pathogenesis and also in the ensuing HTLV-1–associated diseases.

## Disclosures

The authors have no financial conflicts of interest.

## References


1. Proietti, F. A., A. B. Carneiro-Proietti, B. C. Catalan-Soares, and E. L. Murphy. 2005. Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* 24: 6058–6068.
2. Kaplan, J. E., M. Osame, H. Kubota, A. Igata, H. Nishitani, Y. Maeda, R. F. Khabbaz, and R. S. Janssen. 1990. The risk of development of HTLV-I-associated myelopathy/tropical spastic paraparesis among persons infected with HTLV-I. *J. Acquir. Immune Defic. Syndr.* 3: 1096–1101.
3. Murphy, E. L., B. Hanchard, J. P. Figueroa, W. N. Gibbs, W. S. Lofters, M. Campbell, J. J. Goedert, and W. A. Blattner. 1989. Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. *Int. J. Cancer* 43: 250–253.
4. Ohshima, K. 2007. Pathological features of diseases associated with human T-cell leukemia virus type I. *Cancer Sci.* 98: 772–778.
5. Uchiyama, T., J. Yodoi, K. Sagawa, K. Takatsuki, and H. Uchino. 1977. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 50: 481–492.
6. Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. de Thé. 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 326: 407–410.
7. Osame, M., K. Usuku, S. Izumo, N. Ijichi, H. Amitani, A. Igata, M. Matsumoto, and M. Tara. 1986. HTLV-I associated myelopathy, a new clinical entity. *Lancet* 327: 1031–1032.
8. Maloney, E. M., F. R. Cleghorn, O. S. Morgan, P. Rodgers-Johnson, B. Cranston, N. Jack, W. A. Blattner, C. Bartholomew, and A. Manns. 1998. Incidence of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in Jamaica and Trinidad. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 17: 167–170.
9. Maguer-Satta, V., L. Gazzolo, and M. D. Dodon. 1995. Human immature thymocytes as target cells of the leukemogenic activity of human T-cell leukemia virus type I. *Blood* 86: 1444–1452.
10. Osame, M., R. Janssen, H. Kubota, H. Nishitani, A. Igata, S. Nagataki, M. Mori, I. Goto, H. Shimabukuro, R. Khabbaz, et al. 1990. Nationwide survey of HTLV-I-associated myelopathy in Japan: association with blood transfusion. *Ann. Neurol.* 28: 50–56.
11. Ohashi, T., S. Hanabuchi, H. Kato, Y. Koya, F. Takemura, K. Hirokawa, T. Yoshiki, Y. Tanaka, M. Fujii, and M. Kannagi. 1999. Induction of adult T-cell leukemia-like lymphoproliferative disease and its inhibition by adoptive immunotherapy in T-cell-deficient nude rats inoculated with syngeneic human T-cell leukemia virus type 1-immortalized cells. *J. Virol.* 73: 6031–6040.
12. Kannagi, M., T. Ohashi, S. Hanabuchi, H. Kato, Y. Koya, A. Hasegawa, T. Masuda, and T. Yoshiki. 2000. Immunological aspects of rat models of HTLV type 1-infected T lymphoproliferative disease. *AIDS Res. Hum. Retroviruses* 16: 1737–1740.
13. Asquith, B., and C. R. Bangham. 2007. Quantifying HTLV-I dynamics. *Immunol. Cell Biol.* 85: 280–286.
14. Wattel, E., J. P. Vartanian, C. Pannetier, and S. Wain-Hobson. 1995. Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. *J. Virol.* 69: 2863–2868.
15. Matsuoka, M., and K. T. Jeang. 2007. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat. Rev. Cancer* 7: 270–280.

16. Igakura, T., J. C. Stinchcombe, P. K. Goon, G. P. Taylor, J. N. Weber, G. M. Griffiths, Y. Tanaka, M. Osame, and C. R. Bangham. 2003. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* 299: 1713–1716.
17. McDonald, D., L. Wu, S. M. Bohks, V. N. KewalRamani, D. Unutmaz, and T. J. Hope. 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* 300: 1295–1297.
18. Jolly, C., K. Kashefi, M. Hollinshead, and Q. J. Sattentau. 2004. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. *J. Exp. Med.* 199: 283–293.
19. Richardson, J. H., A. J. Edwards, J. K. Cruickshank, P. Rudge, and A. G. Dalgleish. 1990. In vivo cellular tropism of human T-cell leukemia virus type 1. *J. Virol.* 64: 5682–5687.
20. Hanon, E., J. C. Stinchcombe, M. Saito, B. E. Asquith, G. P. Taylor, Y. Tanaka, J. N. Weber, G. M. Griffiths, and C. R. Bangham. 2000. Fratricide among $CD8^+$ T lymphocytes naturally infected with human T cell lymphotropic virus type I. *Immunity* 13: 657–664.
21. Nagai, M., M. B. Brennan, J. A. Sakai, C. A. Mora, and S. Jacobson. 2001. $CD8^+$ T cells are an in vivo reservoir for human T-cell lymphotropic virus type I. *Blood* 98: 1858–1861.
22. Grant, C., K. Barmak, T. Alefantis, J. Yao, S. Jacobson, and B. Wigdahl. 2002. Human T cell leukemia virus type I and neurologic disease: events in bone marrow, peripheral blood, and central nervous system during normal immune surveillance and neuroinflammation. *J. Cell. Physiol.* 190: 133–159.
23. Lehky, T. J., C. H. Fox, S. Koenig, M. C. Levin, N. Flerlage, S. Izumo, E. Sato, C. S. Raine, M. Osame, and S. Jacobson. 1995. Detection of human T-lymphotropic virus type I (HTLV-I) tax RNA in the central nervous system of HTLV-I-associated myelopathy/tropical spastic paraparesis patients by in situ hybridization. *Ann. Neurol.* 37: 167–175.
24. Watabe, K., T. Saida, and S. U. Kim. 1989. Human and simian glial cells infected by human T-lymphotropic virus type I in culture. *J. Neuropathol. Exp. Neurol.* 48: 610–619.
25. Yamada, M., K. Watabe, T. Saida, and S. U. Kim. 1991. Increased susceptibility of human fetal astrocytes to human T-lymphotropic virus type I in culture. *J. Neuropathol. Exp. Neurol.* 50: 97–107.
26. Hoffman, P. M., S. Dhib-Jalbut, J. A. Mikovits, D. S. Robbins, A. L. Wolf, G. K. Bergey, N. C. Lohrey, O. S. Weislow, and F. W. Ruscetti. 1992. Human T-cell leukemia virus type I infection of monocytes and microglial cells in primary human cultures. *Proc. Natl. Acad. Sci. USA* 89: 11784–11788.
27. Kasai, T., H. Ikeda, U. Tomaru, I. Yamashita, O. Ohya, K. Morita, A. Wakisaka, E. Matsuoka, T. Moritoyo, K. Hashimoto, et al. 1999. A rat model of human T lymphocyte virus type I (HTLV-I) infection: in situ detection of HTLV-I provirus DNA in microglia/macrophages in affected spinal cords of rats with HTLV-I-induced chronic progressive myeloneuropathy. *Acta Neuropathol.* 97: 107–112.
28. Ali, A., S. Patterson, K. Cruickshank, P. Rudge, A. G. Dalgleish, and S. C. Knight. 1993. Dendritic cells infected in vitro with human T cell leukaemia/lymphoma virus type-1 (HTLV-1); enhanced lymphocytic proliferation and tropical spastic paraparesis. *Clin. Exp. Immunol.* 94: 32–37.
29. Makino, M., S. Wakamatsu, S. Shimokubo, N. Arima, and M. Baba. 2000. Production of functionally deficient dendritic cells from HTLV-I-infected monocytes: implications for the dendritic cell defect in adult T cell leukemia. *Virology* 274: 140–148.
30. Makino, M., A. Utsunomiya, Y. Maeda, S. Shimokubo, S. Izumo, and M. Baba. 2001. Association of CD40 ligand expression on HTLV-I-infected T cells and maturation of dendritic cells. *Scand. J. Immunol.* 54: 574–581.
31. Hishizawa, M., K. Imada, T. Kitawaki, M. Ueda, N. Kadowaki, and T. Uchiyama. 2004. Depletion and impaired interferon-alpha-producing capacity of blood plasmacytoid dendritic cells in human T-cell leukaemia virus type I-infected individuals. *Br. J. Haematol.* 125: 568–575.
32. Macatonia, S. E., J. K. Cruickshank, P. Rudge, and S. C. Knight. 1992. Dendritic cells from patients with tropical spastic paraparesis are infected with HTLV-1 and stimulate autologous lymphocyte proliferation. *AIDS Res. Hum. Retroviruses* 8: 1699–1706.
33. Jones, K. S., C. Petrow-Sadowski, Y. K. Huang, D. C. Bertolette, and F. W. Ruscetti. 2008. Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of $CD4^+$ T cells. *Nat. Med.* 14: 429–436.
34. Manuel, S. L., T. D. Schell, E. Acheampong, S. Rahman, Z. K. Khan, and P. Jain. 2009. Presentation of human T cell leukemia virus type 1 (HTLV-1) Tax protein by dendritic cells: the underlying mechanism of HTLV-1-associated neuroinflammatory disease. *J. Leukoc. Biol.* 86: 1205–1216.
35. Makino, M., S. Shimokubo, S. I. Wakamatsu, S. Izumo, and M. Baba. 1999. The role of human T-lymphotropic virus type 1 (HTLV-1)-infected dendritic cells in the development of HTLV-1-associated myelopathy/tropical spastic paraparesis. *J. Virol.* 73: 4575–4581.
36. Kampani, K., K. Quann, J. Ahuja, B. Wigdahl, Z. K. Khan, and P. Jain. 2007. A novel high throughput quantum dot-based fluorescence assay for quantitation of virus binding and attachment. *J. Virol. Methods* 141: 125–132.
37. Jain, P., S. L. Manuel, Z. K. Khan, J. Ahuja, K. Quann, and B. Wigdahl. 2009. DC-SIGN mediates cell-free infection and transmission of human T-cell lymphotropic virus type 1 by dendritic cells. *J. Virol.* 83: 10908–10921.
38. Jacobson, S., H. Shida, D. E. McFarlin, A. S. Fauci, and S. Koenig. 1990. Circulating $CD8^+$ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* 348: 245–248.
39. Mostoller, K., C. C. Norbury, P. Jain, and B. Wigdahl. 2004. Human T-cell leukemia virus type I Tax induces the expression of dendritic cell markers associated with maturation and activation. *J. Neurovirol.* 10: 358–371.
40. Ahuja, J., K. Kampani, S. Datta, B. Wigdahl, K. E. Flaig, and P. Jain. 2006. Use of human antigen presenting cell gene array profiling to examine the effect of human T-cell leukemia virus type 1 Tax on primary human dendritic cells. *J. Neurovirol.* 12: 47–59.
41. Ahuja, J., V. Lepoutre, B. Wigdahl, Z. K. Khan, and P. Jain. 2007. Induction of pro-inflammatory cytokines by human T-cell leukemia virus type-1 Tax protein as determined by multiplexed cytokine protein array analyses of human dendritic cells. *Biomed Pharmacother.* 61: 201–208.
42. Jain, P., J. Ahuja, Z. K. Khan, S. Shimizu, O. Meucci, S. R. Jennings, and B. Wigdahl. 2007. Modulation of dendritic cell maturation and function by the Tax protein of human T cell leukemia virus type 1. *J. Leukoc. Biol.* 82: 44–56.
43. Delebecque, F., K. Pramberger, M. C. Prévost, M. Brahic, and F. Tangy. 2002. A chimeric human T-cell lymphotropic virus type 1 with the envelope glycoprotein of Moloney murine leukemia virus is infectious for murine cells. *J. Virol.* 76: 7883–7889.
44. Delebecque, F., C. Combredet, A. S. Gabet, E. Wattel, M. Brahic, and F. Tangy. 2005. A chimeric human T cell leukemia virus type I bearing a deltaR Moloney-murine leukemia virus envelope infects mice persistently and induces humoral and cellular immune responses. *J. Infect. Dis.* 191: 255–263.
45. Potash, M. J., W. Chao, G. Bentsman, N. Paris, M. Saini, J. Nitkiewicz, P. Belem, L. Sharer, A. I. Brooks, and D. J. Volsky. 2005. A mouse model for study of systemic HIV-1 infection, antiviral immune responses, and neuroinvasiveness. *Proc. Natl. Acad. Sci. USA* 102: 3760–3765.
46. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. In vivo depletion of $CD11c^+$ dendritic cells abrogates priming of $CD8^+$ T cells by exogenous cell-associated antigens. *Immunity* 17: 211–220.
47. Yamano, Y., M. Nagai, M. Brennan, C. A. Mora, S. S. Soldan, U. Tomaru, N. Takenouchi, S. Izumo, M. Osame, and S. Jacobson. 2002. Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific $CD8^+$ T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). *Blood* 99: 88–94.
48. Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402–408.
49. Huleatt, J. W., and L. Lefrançois. 1995. Antigen-driven induction of CD11c on intestinal intraepithelial lymphocytes and $CD8^+$ T cells in vivo. *J. Immunol.* 154: 5684–5693.
50. Lin, Y., T. J. Roberts, V. Sriram, S. Cho, and R. R. Brutkiewicz. 2003. Myeloid marker expression on antiviral $CD8^+$ T cells following an acute virus infection. *Eur. J. Immunol.* 33: 2736–2743.
51. Probst, H. C., and M. van den Broek. 2005. Priming of CTLs by lymphocytic choriomeningitis virus depends on dendritic cells. *J. Immunol.* 174: 3920–3924.
52. Kassim, S. H., N. K. Rajasagi, X. Zhao, R. Chervenak, and S. R. Jennings. 2006. In vivo ablation of CD11c-positive dendritic cells increases susceptibility to herpes simplex virus type 1 infection and diminishes NK and T-cell responses. *J. Virol.* 80: 3985–3993.
53. Knight, S. C., S. E. Macatonia, K. Cruickshank, P. Rudge, and S. Patterson. 1993. Dendritic cells in HIV-1 and HTLV-1 infection. *Adv. Exp. Med. Biol.* 329: 545–549.
54. Fang, J., S. Kushida, R. Feng, M. Tanaka, T. Kawamura, H. Abe, N. Maeda, M. Onobori, M. Hori, K. Uchida, and M. Miwa. 1998. Transmission of human T-cell leukemia virus type 1 to mice. *J. Virol.* 72: 3952–3957.
55. Feng, R., M. Tanaka, H. Abe, N. Arashi, B. Sun, K. Uchida, and M. Miwa. 1999. Human T-cell leukemia virus type 1 can infect a wide variety of cells in mice. *Jpn. J. Cancer Res.* 90: 48–54.
56. Kushida, S., N. Maeda, J. Fang, K. Uchida, and M. Miwa. 1997. Establishment of HTLV-1 carrier mice by injection with HTLV-1-producing T cells. *Leukemia* 11(Suppl 3): 260–262.
57. Denesvre, C., P. Sonigo, A. Corbin, H. Ellerbrok, and M. Sitbon. 1995. Influence of transmembrane domains on the fusogenic abilities of human and murine leukemia retrovirus envelopes. *J. Virol.* 69: 4149–4157.
58. Li, Q. X., D. Camerini, Y. Xie, M. Greenwald, D. R. Kuritzkes, and I. S. Chen. 1996. Syncytium formation by recombinant HTLV-II envelope glycoprotein. *Virology* 218: 279–284.
59. Nagy, K., P. Clapham, R. Cheingsong-Popov, and R. A. Weiss. 1983. Human T-cell leukemia virus type I: induction of syncytia and inhibition by patients' sera. *Int. J. Cancer* 32: 321–328.
60. Sommerfelt, M. A., B. P. Williams, P. R. Clapham, E. Solomon, P. N. Goodfellow, and R. A. Weiss. 1988. Human T cell leukemia viruses use a receptor determined by human chromosome 17. *Science* 242: 1557–1559.
61. Bennett, C. L., and B. E. Clausen. 2007. DC ablation in mice: promises, pitfalls, and challenges. *Trends Immunol.* 28: 525–531.
62. Tateno, M. 1987. [Rat lymphoid cell lines producing human T cell leukemia virus-I]. *Hokkaido Igaku Zasshi* 62: 74–81.