ILAR Journal, 2016, Vol. 57, No. 1, 3–11

doi: 10.1093/ilar/ilv045
Article

Animals Models of Human T Cell Leukemia Virus Type I Leukemogenesis

Stefan Niewiesk

Stefan Niewiesk, DVM, PhD, is a professor in the Department of Veterinary Biosciences in the College of Veterinary Medicine at the Ohio State University in Columbus, Ohio.

Address correspondence and reprint requests to Dr. Stefan Niewiesk, Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, 1925 Coffey Road, Columbus OH 43210 or email niewiesk.1@osu.edu.

Abstract

Infection with human T cell leukemia virus type I (HTLV-I) causes adult T cell leukemia (ATL) in a minority of infected individuals after long periods of viral persistence. The various stages of HTLV-I infection and leukemia development are studied by using several different animal models: (1) the rabbit (and mouse) model of persistent HTLV-I infection, (2) transgenic mice to model tumorigenesis by HTLV-I specific protein expression, (3) ATL cell transfers into immune-deficient mice, and (4) infection of humanized mice with HTLV-I. After infection, virus replicates without clinical disease in rabbits and to a lesser extent in mice. Transgenic expression of both the transactivator protein (Tax) and the HTLV-I bZIP factor (HBZ) protein have provided insight into factors important in leukemia/lymphoma development. To investigate factors relating to tumor spread and tissue invasion, a number of immune-deficient mice based on the severe combined immunodeficiency (SCID) or non-obese diabetic/SCID background have been used. Inoculation of adult T cell leukemia cell (lines) leads to lymphoma with osteolytic bone lesions and to a lesser degree to leukemia development. These mice have been used extensively for the testing of anticancer drugs and virotherapy. A recent development is the use of so-called humanized mice, which, upon transfer of $CD34^+$ human umbilical cord stem cells, generate human lymphocytes. Infection with HTLV-I leads to leukemia/lymphoma development, thus providing an opportunity to investigate disease development with the aid of molecularly cloned viruses. However, further improvements of this mouse model, particularly in respect to the development of adaptive immune responses, are necessary.

Key words: adult T cell leukemia; CD4 T cell; HTLV-I BZIP factor; human T cell leukemia virus; immune-deficient mice; lymphoma/leukemia; transactivator protein; transgenic mice

Diseases Induced by Human T Cell Leukemia Virus Type I Infection in Humans

Approximately 5 to 20 million people worldwide are infected with human T cell leukemia virus type I (HTLV-I) (de Thé and Bomford 1993; Gessain and Cassar 2012). The highest prevalence of infection is found in Japan, the Carribean, Africa, South America, and the Pacific islands (Proietti et al. 2005). Infection with HTLV-I leads to life-long persistence in humans. After decades of infection, a minority of individuals (an estimated 2–5%) develops clinical symptoms such as HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) or adult T cell leukemia (ATL) (Ishitsuka and Tamura 2014). HAM/TSP is a chronic inflammatory disease of the spinal cord that results in weakness of the legs or paraparesis. The spinal cord as such is not infected with HTLV-I but is infiltrated with HTLV-I–infected and –uninfected T cells, which are thought to cause damage to the nerve tissue. Adult T cell leukemia is caused by the transformation of CD4T cells into leukemic cells by HTLV-I infection and leads to an aggressive and ultimately untreatable leukemia/lymphoma (Ishitsuka and Tamura 2014). Because a chronically active immune response against the virus is found in infected asymptomatic individuals, the strength of immune surveillance is thought be an important factor in ATL development (Arnulf et al. 2004; Kannagi et al. 2005;

Masamoto et al. 2015; Matsuoka and Jeang 2007) (the other contributing factor being the transformation of leukemic cells). ATL is usually a highly aggressive non-Hodgkin's lymphoma (Ishitsuka and Tamura 2014; Tsukasaki and Tobinai 2014) with no characteristic histologic appearance except for a diffuse pattern of leukemic cells and a mature T cell phenotype. $CD4^+$ leukemic T cells often express CCR4 (Yoshie et al. 2002), CD25 (alpha chain of the interleukin 2 receptor) (Shimoyama 1991), and FoxP3 (a marker of regulatory T cells) (Roncador et al. 2005). Four types of adult T cell leukemia have been defined by the Japan Clinical Oncology Group in HTLV-I–infected patients: acute, lymphomatous, chronic, and smoldering leukemia (Shimoyama 1991). The defining features of acute leukemia are high white blood cell (WBC) counts with lymphocytosis, atypical lymphocytes and eosinophilia, hepatosplenomegaly, lymphadenopathy, bone marrow infiltration, variable lytic bone lesions resulting in hypercalcemia, and skin lesions. In addition, patients are susceptible to opportunistic pathogens such as *Pneumocystis jirovecci* (Ishitsuka and Tamura 2014). The prognosis of acute leukemia is poor, and median survival is less than 1 year despite chemotherapy. The other types of ATL present with fewer clinical signs and have prolonged time of survival (Ishitsuka and Tamura 2014; Tobinai 2009; Tsukasaki and Tobinai 2014). In lymphomatous leukemia, cutaneous lesions and lymphadenopathy are common; in chronic leukemia, an increase in WBCs and a skin rash is observed; and in smoldering leukemia, only a few malignant cells are observed in the blood stream (Ishitsuka and Tamura 2014).

Animal Models for HTLV-I and Related Viruses

HTLV-I is a member of the deltaretroviruses. This virus family also includes bovine leukemia virus (BLV) and simian T cell leukemia virus (STLV). Because of its high economic impact, BLV has been studied in some detail using the sheep model (Aida et al. 2013; Florins et al. 2008). Although the virus has a number of similarities to HTLV-I on the molecular level, it differs from HTLV-I in that it does not cause neurological disease and induces B cell rather than T cell leukemia. Simian T cell leukemia virus has been reported to induce T cell leukemia in various primates similar to HTLV-I in humans but has not been used to model virus-induced leukemia in primates (Lapin and Yakovleva 2014; Panfil et al. 2013). The various stages of HTLV-I infection and leukemia development are studied by using several different animal models: (1) the rabbit (and mouse) model of persistent HTLV-I infection, (2) transgenic mice to model tumorigenesis by HTLV-I–specific protein expression, (3) ATL cell transfers into immune-deficient mice, and (4) infection of humanized mice with HTLV-I. These animal models are discussed herein. In addition, a model of HTLV-I–induced paraparesis has been established in WKA rats. Because HTLV-I infection relies on cell-to-cell transmission, animals have to be infected by injection of virus-producing cell lines. If these cell lines are tumorigenic on their own, they have to be inactivated by either mitomycin treatment or irradiation. Injection of HTLV-I–infected cell lines into WKA rats led, in one study, to paraparesis in more than half of infected rats after a few months (Kushida et al. 1994). However, in a different study, the incidence of paraparesis was lower and not necessarily linked to HTLV-I infection (Sun et al. 1999). In contrast with humans, in rats HTLV-I provirus is found in peripheral blood mononuclear cells and the spinal cord tissue by polymerase chain reaction (Kushida et al. 1993, 1994; Mizusawa et al. 1994).

Asymptomatic HTLV-I Infection in Small Laboratory Animals

HTLV-I Infection of Mice

The task of developing an animal model for HTLV-I infection encounters the common problem that mice are often not susceptible to human pathogens. In tissue culture, HTLV-I grows in human but not mouse cells although infection or overexpression of the transactivator protein (Tax) will lead to immortalization of rodent fibroblasts (Grassmann et al. 2005). In mice, inoculation with HTLV-I–infected cells leads to integration of provirus into lymphoid cells. Integrated provirus persists for months, but active viral replication is not detectable (Fang et al. 1998; Kushida et al. 1997; Tanaka et al. 2001). A chimeric HTLV-I virus expressing the envelope gene of Moloney murine leukemia virus in place of its own envelope protein is able to better infect and replicate in the mouse (Delebecque et al. 2005). In contrast with HTLV-I, this chimeric virus infects organs such as the brain, lung, and spinal cord. After depletion of dendritic cells, infection with this recombinant virus is reduced. This indicates that dendritic cells play an important role in the early infection and pathogenesis of disease (Rahman et al. 2010). In addition, it was shown that NFκB plays an important role in virus spread and replication because, in mice with a defect in NFκB activation due to an amino acid substitution in the carboxy-terminal interaction domain of NF-κB–inducing kinase, virus spread and replication is reduced (Nitta et al. 2008). However, the HTLV-I envelope protein is thought to play an important role in cell tropism, viral spread, and disease development, and therefore infection with the chimeric virus does not fully reflect infection in humans.

In an alternate approach, immune-deficient (NOG) mice were injected with human peripheral lymphocytes from HTLV-I–negative individuals. These lympyhocytes were infected with HTLV-I through injection of an HTLV-I–producing cell line (MT2), which was inactivated through mitomycin treatment. Both CD4 and CD8T cell populations were found to be infected. Infection of human lymphocytes in this model could be prevented by an anti–HTLV-I antibody (Saito et al. 2014) and a prophylactive treatment with the reverse-transcriptase blockers azidothymidine and tenofovir (Miyazato et al. 2006).

HTLV-I Infection of Rabbits

As an alternate model for asymptomatic infection, rabbits have been used extensively because of the ease and consistency of viral transmission and infection in this species. Infectivity in rabbits was first demonstrated in the mid-1980s by intravenous inoculation of a rabbit lymphocyte cell line (Ra-1) that had been infected with HTLV-I through coculture with the HTLV-I–infected human MT-2 cell line (Akagi et al. 1985; Miyoshi et al. 1985). Early studies in rabbits identified the routes of viral transmission (e.g., blood, semen, milk) (Hirose et al. 1988; Iwahara et al. 1990; Kataoka et al. 1990; Kotani et al. 1986; Uemura et al. 1986, 1987). The rabbit model has provided important information about the immune responses during HTLV-I infection. Early studies defined methods to detect the sequential development of antibodies against different viral proteins and HTLV-I proviral DNA in infected tissues (Cockerell et al. 1990). Immunization of rabbits with synthetic peptides verified immunodominant epitopes of the viral envelope protein (Env) (Lal et al. 1991; Tanaka et al. 1991) and also defined regions of Env important for antibody-dependent cell-mediated cytotoxicity (Chen et al. 1991). Subsequently, it was demonstrated that peptide immunization with

amino acids 190 to 199 of the Env protein could protect rabbits from HTLV-I infection (Tanaka et al. 1994). More complex synthetic peptides, which use chimeric constructs that mimic native viral proteins, have also been generated and tested in the rabbit model (Conrad et al. 1995; Frangione-Beebe et al. 2000). Infectious molecular clones of HTLV-I were first developed in the mid-1990s (Derse et al. 1995; Kimata et al. 1994; Zhao et al. 1995). These molecular clones were used to immortalize human peripheral blood mononuclear cells to create the ACH.2 cell line, which was then used to infect rabbits (Collins et al. 1996). Subsequently, recombinant HTLV-I viruses with mutations in the open reading frames encoding the HTLV-I accessory proteins p12, p13, and p30 were generated (Robek et al. 1998). These mutants were then inoculated into rabbits to demonstrate the necessity of these accessory proteins for establishment of infection and maintenance of proviral loads. Recombinant HTLV-I viruses with selected mutations have been used to test the in vivo functional properties of HTLV-I p12, p13, p30, Rex, and Env (Arnold et al. 2006; Bartoe et al. 2000; Collins et al. 1998; Hiraragi et al. 2006; Silverman et al. 2004, 2005).

Leukemia Induction in Transgenic Mice Expressing Tumor-Inducing/-Maintaining Genes

Tax-Transgenic Mice

Because infection of both mice and rabbits leads only to asymptomatic infection, additional animal models were developed that could be used to investigate adult T cell leukemia. One approach is to express the oncogenic proteins of a virus transgenically in mice. In the case of HTLV-I, Tax is thought to be the major oncogenic protein of the virus. This is based on the ability of Tax to activate a variety of cellular promotors leading to cell activation and replication, which is thought to lead to accumulation of mutations in the genome with subsequent cell transformation (Matsuoka and Jeang 2007). The second line of evidence is the ability of Tax to transform rodent fibroblasts in vitro (Grassmann et al. 2005). To test the tumorigenic potential of Tax in vivo, large numbers of transgenic mice have been produced. All of these mice express the Tax protein but differ in the promoter usage. Expression of Tax has resulted in different diseases depending on the transgenic mouse line. Typical for all of them is the overexpression of Tax in comparison with natural infection and the long time to develop disease (typically 6 to 20 months). Expression of Tax in most transgenic mice does not lead to leukemia/lymphoma induction but to other less typical manifestations of HTLV-I infection. In a number of transgenic mouse lines, the long terminal repeat (LTR), which is the natural virus promoter, has been used. In a study by Bieberich and colleagues (1993) it was shown that the HTLV-I LTR can be activated by Tax in the mouse not only in lymphocytes but also in a wide variety of tissues such as muscle, bone, skin, nerve, and salivary glands. This activation pattern might explain the varied results obtained with transgenic mice using the LTR. In some mouse lines, the use of the LTR promoter leads to the generation of transgenic mice that developed neurofibroma and died early (Hinrichs et al. 1987; Nerenberg et al. 1987). Other groups reported that LTR promoter–driven Tax expression lead to the development of Sjögren's syndrome (Green et al. 1989) or skeletal abnormalities (Ruddle et al. 1993). (Sjögren's syndrome is an autoimmune disease during which the salivary and lacrimal glands are attacked by immune cells [Borchers et al. 2003]). Other mice with LTR promoter–driven Tax expression developed arthritis (Habu et al. 1999). A puzzling case of arthritis induction was seen after the use of Tax driven by the CD4 promoter, which theoretically should have led to leukemia induction (Habu et al. 1999). An inducible metallothionein promoter supposed to regulate Tax expression after induction with heavy metals was found to be leaky. Expression of Tax in these mice leads to arthropathy (Saggioro et al. 1997). So far, only two promoters have led to the development of leukemia/lymphoma: the granzyme B promoter (GzmB, which is activated in natural killer [NK] cells and T cells) and the lymphocyte-specific protein tyrosine kinase $p56^{lck}$ (*lck*) promoter. Of the latter, two versions exist: the proximal promoter drives gene expression in thymocytes, whereas the distal promoter drives gene expression in mature T cells. Mice with Tax expression driven by GzmB resemble the situation in ATL patients in that tumors are associated with inflammation and neutrophila (Grossman et al. 1995). GzmB-Tax mice have a 100% penetrance of $CD16/32^+$ $B220^+$ $CD3c^+$ large granular lymphocytic leukemia/lymphoma involving mesenteric and peripheral lymph nodes and spleen. These mice display high rates of peripheral disease (i.e., skin or subcutaneous tumors), lytic bone disease, and hypercalcemia. If osteoclast activity is inhibited, either by application of zoledronic acid or transgenic expression of osteoprotegerin, mice do not develop osteolytic bone lesions and have fewer soft tissue tumors (Gao et al. 2005). This model was expanded by crossing GzmB-Tax mice with mice expressing luciferase under the LTR promoter so that Tax-expressing tumors can be detected by bioluminescence. It was found that subcutaneous tumor formation was preceded by development of microscopic intraepithelial lesions (Rauch et al. 2009). When these mice were crossed with mice carrying a deletion in the interleukin 15 (IL-15) gene, tumor development and accelerated mortality were observed, which indicated a role for antitumor immunity for IL-15. The absence of IL-15 resulted in upregulation of (interleukin 1α) IL-1α and IL-1α–dependent genes. This gene upregulation is causally linked to tumor development because treatment with an IL-1α–neutralizing antibody reduces tumor incidence (Rauch et al. 2014).

The other promoter that leads to development of leukemia and lymphoma in mice expressing Tax is the *lck* promoter. Mice that expressed Tax under the control of the proximal *lck* promoter developed large-cell lymphomas and leukemia (Hasegawa et al. 2006). The leukemic cells had a pre–T cell phenotype ($CD4^-$, $CD8^-$, $CD44^+$, $CD25^+$, cytoplasmic $CD3^+$). Leukemic mice were immune compromised (similar to ATL patients) and developed pulmonary infection with *P. jirovecci*. Mice that expressed Tax under the control of the distal *lck* promoter were reported (Ohsugi, Kumasaka, Okada, and Urano 2007) to develop leukemia and lymphoma. The leukemic T cells were $CD4^+$, $CD8^+$, or $CD4^+/CD8^+$. In addition, some of these mice developed arthropathy and histiocytic sarcoma (Ohsugi et al. 2013).

HBZ-Transgenic Mice

The sole role of Tax in leukemia development has been questioned because in a number of ATL cells Tax is no longer expressed (Matsuoka and Jeang 2007). A few years ago, an additional protein from an open reading frame in the negative orientation of the HTLV-I genome was identified as HTLV-I bZIP factor (HBZ). There is the suggestion that this protein is also an inducer of leukemia and/or a maintenance factor (Mesnard et al. 2006). In transgenic mice, the expression of HBZ under the mouse CD4 promoter leads to mild leukemia and lymphoma in skin, lung, and the intestine (Satou et al. 2011). When double-transgenic mice that expressed both HBZ and Tax under the

mouse CD4 promoter were produced, no additive effect was observed in terms of leukemia and lymphoma development or in terms of reduction of survival (Zhao et al. 2014). One possible explanation for the lack of synergy between Tax and HBZ might be that mice expressing Tax only did not develop disease. In another mouse model, the expression of the envelope protein as well as Tax and HBZ (env-pX) under the control of LTR led to arthritis development (Iwakura et al. 1991). In ATL patients, an increase in regulatory T cells, as determined by the expression of the FoxP3 protein (Karube et al. 2004), has been detected. In HBZ-transgenic mice, HBZ has been reported to stimulate the production of interferon γ (IFNγ), thus leading to increased inflammation and dermatitis (Mitagami et al. 2015). The same group has also reported HBZ to increase the expression of FoxP3 and to decrease the production of IFNγ specifically in T helper cells. This leads to a higher-than-normal number of regulatory T cells (Satou et al. 2011) and increased susceptibility of these mice toward infection with herpes simplex virus type 2 or Listeria monocytogenes (Sugata et al. 2012).

Adult T Cell Leukemia Cell (Line) Transfer into Immune-Deficient Mice

In transgenic mice, the viral protein is not expressed in the context of the virus and is often overexpressed compared with viral infection. In addition, specifically the expression of Tax leads to a variety of disease manifestations that do not clearly correlate with human disease. To develop animal models in which the spread and tissue invasion of ATL cells can be tested, immune-deficient rodents have been used.

Athymic Rats

In a model of athymic F344/N rats, inoculation of an HTLV-I immortalized rat T cell line led to development of leukemia and lymphoma. When congenic rats were immunized with Tax peptides they developed CD4 and CD8T cells, which protected against lymphoma development upon T cell transfer (Hanabuchi et al. 2001; Nomura et al. 2004).

Immune-Deficient Mice

To develop mouse models where the spread and tissue invasion of ATL cells can be tested, four mouse strains with differing degrees of immune deficiency have been utilized. All mouse strains are based on the immune-deficient severe combined immunodeficiency (SCID) mouse. SCID mice do not have functional B cell or T cells but have residual NK cell activity, which interferes sometimes with establishment of tumors. Non obese diabetic (NOD)/SCID mice do not have a functional B cell or T cell response, and the activity of their NK cells is reduced (Shultz et al. 1995). In NOD/SCID$^{\beta 2m-/-}$ mice, the β2-microglobulin gene is deleted, and in consequence the NK cell activity is completely abolished (Koller and Smithies 1989). In NOD/SCID$^{IL2\text{-}R\gamma\ -/-}$ mice, the gene for the gamma chain of the interleukin 2 (IL-2) receptor is either truncated (NOG) or deleted (NSG), which, in addition to the other immune deficiencies, leads to a defect in the inability of dendritic cells to secrete IFNγ upon stimulation (Ohbo et al. 1996).

SCID Mice

The SCID mouse has been a successful model to investigate the proliferative and tumorigenic potential of ATL cell lines (Feuer et al. 1993; Ishihara et al. 1992; Kondo et al. 1993; Ohsugi et al. 1994). In some studies, leukemic cells from Tax-transgenic mice (with the *lck* promoter) were used to induce leukemia and lymphoma in SCID mice (Ishitsuka and Tamura 2014). In these mice, cytokines, growth factors, and adhesion molecules were analyzed for their role in leukemogenesis, and tumor necrosis factor α (TNF-α), platelet-derived growth factor subunit B (homodimer), soluble intercellular adhesion molecule 1, soluble vascular cell adhesion protein 1 were identified as factors that may contribute to high levels of organ infiltration in this model (Watters et al. 2010). After transfer of splenic leukemic cells from Tax-transgenic mice it has been possible to identify cancer stem cells. These cells expressed CD117^{+} and low levels of Tax, Notch1, and Bmi1, which suggests that they are pre–T cells or early hematopoetic progenitor cells. In a naive mouse, 10^2 cancer stem cells were sufficient to repopulate the leukemic population. In contrast, the transfer of 10^2 splenic leukemic cells did not result in leukemia (Yamazaki et al. 2009). In this model, the protective effect of a synthetic retinoid (ST19260), which induces apoptosis in tumor cells and decreases Tax expression (El Hajj et al. 2014), was demonstrated.

For inoculation with human cells, primary leukemic cells from patients, lymphocytes transformed in vitro by HTLV-I (which are IL-2 dependent), or HTLV-I immortalized cell lines have been tested in SCID mice. Inoculation of primary ATL cells or lymphocytes transformed in vitro by HTLV-I usually does not lead to tumor growth (Liu et al. 2002; Parrula et al. 2009). However, in some instances it has been possible to accomplish growth of leukemic cells by depleting NK cells with antibody treatment and by IL-2 supplementation through daily injections (Kondo et al. 1993). Most human HTLV-I immortalized cell lines lead to development of lymphomas, and tumor cells recovered from these mice retain the phenotypic and genotypic characteristics of the original tumor cell line inoculate (Feuer et al. 1993; Imada et al. 1995; Kondo et al. 1993). Interestingly, HTLV-I–infected cell lines of nonleukemic origin are not tumorigenic in SCID mice (Feuer et al. 1995; Imada et al. 1995; Uchiyama 1996) unless NK cell activity has been suppressed by sublethal irradiation or by treatment of animals with antiserum, which transiently abrogates NK activity (anti–asialo-GM1) (Feuer et al. 1995). In addition to leukemia/lymphoma development after inoculation of ATL cell lines, SCID mice were also shown to develop humoral hypercalcemia of malignancy, which is an important clinical sign in ATL patients (Richard et al. 2001). An interesting age effect on the outcome of leukemia development was observed in neonatal SCID mice. After inoculation with ATL cells, adult SCID mice developed leukemia, and animals became moribund over time. When the same cell lines were inoculated into neonatal animals, they succumbed to disease much faster (Ohsugi et al. 2004).

NOD/SCID–Based Mouse Strains

Because of the inhibitory effect of NK cells on leukemia and lymphoma development, most recent studies have been performed in the NOD/SCID mouse and its derivatives. It could be demonstrated that the higher the degree of immune deficiency of the mouse strain the shorter the time to development of leukemia and lymphoma (Parrula et al. 2009). A problem for the study of ATL has been that most cell lines do not spread systemically but grow either subcutaneously after subcutaneous inoculation or in the abdominal cavity after intraperitoneal injection (Dewan et al. 2003; Liu et al. 2002; Ohsugi et al. 2004; Parrula et al. 2009; Richard et al. 2001; Takaori-Kondo et al. 1998). A notable exception is a cell line that was derived from primary ATL cells called MET-1 that metastatizes systemically. In contrast with other cell lines, these cells express adhesion factors CD11a (LFA-1α) and CD49d (VLA-4α) and produce or induce the

expression of matrix metalloproteinases 1, 2, 3, and 9, which serve as tissue invasion factors (Parrula et al. 2009). The MET-1 cell line has been used to test an antibody specific for CD25 (interleukin 2 receptor α) for its antileukemic activity (Zhang et al. 2003). After injection of the antibody, survival of MET-1–inoculated mice was significantly increased (Zhang et al. 2003). The protective effect could be increased by combination with a histone deacetylase inhibitor (depsipeptide) (Chen et al. 2009) or the cytotoxic drug 9-aminoacridine (Ju et al. 2014). The MET-1 NOD/SCID model was also used to test measles virus virotherapy. After inoculation of measles vaccine virus, a rapid regression of leukemia/lymphoma was observed. This effect was more effective at higher levels of tumor burden than the aforementioned antibody treatments (Parrula et al. 2011). Using different ATL lines, it was shown that measles virotherapy works best if the respective tumor line does not express IFNα, suggesting that it may be beneficial to test interferon α (IFNα) expression of tumors in patients (Parrula et al. 2014). Although tumor reduction in mice was effective, mice still eventually became cachectic and moribund. This condition was correlated with interleukin 6 (IL-6) production by both the human tumor cells and mouse cells (Parrula et al. 2011).

NOD/SCID mice have also been used to test drugs against HTLV-I infection and tumor formation. The NFκb inhibitor dehydroxymethylepoxyquinomicin (Ohsugi, Kumasaka, Okada, Ishida et al. 2007) and Bay11-7082 (Dewan et al. 2003), an HSP90 inhibitor (17-DMAG [Ikebe et al. 2013]), a Bcl-2 family inhibitor (ABT-737 [Ishitsuka et al. 2012]), and a histone deacetylase inhibitor (AR42 [Zimmerman et al. 2011]) all proved to be effective in reducing tumor burden. The NOD/SCID mouse/ATL model was improved by the use of bioluminescent ATL cells for the purpose of monitoring in vivo tumor progression. Combination therapy with the proteasome inhibitor PS-341 and an osteoclast inhibitor, zoledronic acid, virtually eliminated propagation of ATL cells in the mouse model and prevented the development of humoral hypercalcemia of malignancy in NOD/SCID mice (Shu et al. 2007). This was accompanied by a significant decrease in PTHrP and MIP-1α levels (expressed at high levels by ATL cells), suggesting that these cytokines play an important role in the development of humoral hypercalcemia of malignancy in ATL.

Study of Leukemia/Lymphoma Development in Humanized Mice after HTLV-I Infection

The ATL xenograft models and the transgenic mouse models do not address how virus infection induces and maintains the development of adult T cell leukemia. Ideally, a mouse could be used that has a human immune system, can be infected with HTLV-I, and develops leukemia/lymphoma. Recent developments in immune-deficient mice have led to so-called humanized mouse models. After injection of human $CD34^+$ umbilical cord stem cells (HUSC), human lymphocytes develop in NSG and NOG mice. Although the lymphocytes are phenotypically normal, they are not functional in respect to the adaptive immune response. However, parts of their immune system, including neutrophils and macrophages, are fully functional. If human umbilical cord stem cells are injected into mice that have received a transplant of fetal liver and thymus, they generate adaptive immune responses that vary in effectiveness. Currently, methodological improvements (transgenic expression of cytokines and others) to these mouse models are being tested, with the goal of improving adaptive immune responses. However, from the animal model perspective, the lack of an adaptive immune response that might interfere with virus replication and leukemogenesis might be an advantage.

Humanized mice have been used for the development of adult T cell leukemia after infection with HTLV-I (Villaudy et al. 2011). Mice were injected with HUSC, and resulting lymphocytes were infected by the injection of irradiated MT-2 cells (Villaudy et al. 2011). The authors reported a leukemia incidence of more than 95%. Interestingly, the proportion of $CD4^+$ and $CD4^+/CD8^+$ T cells was equally high. Macroscopically, enlarged lymph nodes (10 of 16 mice), enlarged thymus (2 of 16 mice), and enlarged spleens (2 of 16 mice) were observed. Histological detection of leukemic cells was reported in spleen and thymus. In patients infected with HTLV-I, the population of infected cells is polyclonal. In patients with adult T cell leukemia, the T cell population changes toward an oligoclonal (and sometimes monoclonal) cell population. In this mouse model, an increase in viral loads was determined as infection progressed and an increase in the oligoclonality index was found. These data are interesting because they indicate that outgrowth of oligoclonal leukemic cells can occur in the absence of a selecting immune response. Another study has tried to address two findings from the intrahepatic injection of HUSC into immune-deficient mice. One is the lack of antigen-specific immune responses, and the other one is the finding that, after intrahepatic injection, mainly T cells develop, which over time make up the majority of cells in the lymphocyte population. In this study, NSG mice were injected intratibially with $CD133^+$ stem cells (Tezuka et al. 2014). $CD133^+$ stem cells are thought to preceed $CD34^+$ stem cells and are able to differentiate into other cell types than lymphocytes, too. Thus it might be possible that human mesenchymal cells, which subsequently support the growth of human B lymphocytes, are generated in the bone marrow. After injection of $CD133^+$ stem cells, a more balanced B cell to T cell ratio developed in these mice, which remained stable for up to 8 months. After infection with HTLV-I, leukemia and lymphoma developed similar to mice injected with $CD34^+$ stem cells. It was possible to detect HTLV-I–specific antibodies and T cells specific for HTLV-I–derived peptides. In the blood of these animals, IL-6, IL-8, IL-10, IL-12, IL-13, IFNγ, TNFα, granulocyte-macrophage colony-stimulating factor, and chemokine (C-C motif) ligand 4 were elevated (Tezuka et al. 2014). Although these immune responses were clearly detectable, they were not sufficient to noticeably increase survival time compared with mice that displayed no immune responses.

Conclusions

In the quest to understand the development of leukemia and lymphoma induced by infection with HTLV-I, multiple animal models have been developed. This indicates that it has been difficult to find the all-encompassing model and that for different aspects of infection—transformation and leukemogenesis, as well as tissue invasion—different models had to be utilized. From a large body of work it seems that the rabbit is the most useful model to study chronic asymptomatic HTLV-I infection. Tax- and also HBZ-transgenic mice are useful tools to study the contribution of these proteins, but the most effective promoter has to be used to express the proteins in lymphocytes and more specifically in CD4T lymphocytes. In respect to the transfer of ATL cell lines into immune-deficient mice, the development of highly immune-deficient mouse strains (NOG and NSG) has been very beneficial, as has been the use of systemically spreading cell lines (MET-1). The use of humanized mice for HTLV-I research is still a nascent field with a lot of promise. It is currently possible to infect these mice and observe leukemia/lymphoma development in a few months. In the future, the continued development of these mice should enable the

investigation of the adaptive immune response, thus providing a tool for developing vaccines against an infectious cancer in this animal model.

Acknowledgments

Stefan Niewiesk is supported by the National Cancer Institute, National Institutes of Health through P01CA100730 "Retrovirus Models of Cancer."

References

Aida Y, Murakami H, Takahashi M, Takeshima SN. 2013. Mechanisms of pathogenesis induced by bovine leukemia virus as a model for human T-cell leukemia virus. Front Microbiol 4:328.

Akagi T, Takeda I, Oka T, Ohtsuki Y, Yano S, Miyoshi I. 1985. Experimental infection of rabbits with human T-cell leukemia virus type 1. Jpn J Cancer Res 76:86–94.

Arnold J, Yamamoto B, Li M, Phipps AJ, Younis I, Lairmore MD, Green PL. 2006. Enhancement of infectivity and persistence in vivo by HBZ, a natural antisense coded protein of HTLV-1. Blood 107:3976–3982.

Arnulf B, Thorel M, Poirot Y, Tamouza R, Boulanger E, Jaccard A, Oksenhendler E, Hermine O, Pique C. 2004. Loss of the ex vivo but not the reinducible $CD8^+$ T-cell response to Tax in human T-cell leukemia virus type 1-infected patients with adult T-cell leukemia/lymphoma. Leukemia 18:126–132.

Bartoe JT, Albrecht B, Collins ND, Robek MD, Ratner L, Green PL, Lairmore MD. 2000. Functional role of pX open reading frame II of human T-lymphotropic virus type 1 in maintenance of viral loads in vivo. J Virol 74:1094–1100.

Bieberich CJ, King CM, Tinkle BT, Jay G. 1993. A transgenic model of transactivation by the Tax protein of HTLV-I. Virology 196:309–318.

Borchers AT, Naguwa SM, Keen CL, Gershwin ME. 2003. Immunopathogenesis of Sjogren's syndrome. Clin Rev Allergy Immunol 25:89–104.

Chen J, Zhang M, Ju W, Waldmann TA. 2009. Effective treatment of a murine model of adult T-cell leukemia using depsipeptide and its combination with unmodified daclizumab directed toward CD25. Blood 113:1287–1293.

Chen YM, Zhang XQ, Dahl CE, Samuel KP, Schooley RT, Essex M, Papas TS. 1991. Delineation of type-specific regions on the envelope glycoproteins of human T cell leukemia viruses. J Immunol 147:2368–2376.

Cockerell GL, Lairmore M, De B, Rovnak J, Hartley TM, Miyoshi I. 1990. Persistent infection of rabbits with HTLV-I: Patterns of anti-viral antibody reactivity and detection of virus by gene amplification. Int J Cancer 45:127–130.

Collins ND, Newbound GC, Albrecht B, Beard JL, Ratner L, Lairmore MD. 1998. Selective ablation of human T-cell lymphotropic virus type 1 p12I reduces viral infectivity in vivo. Blood 91:4701–4707.

Collins ND, Newbound GC, Ratner L, Lairmore MD. 1996. In vitro $CD4^+$ lymphocyte transformation and infection in a rabbit model with a molecular clone of human T-cell lymphotrophic virus type 1. J Virol 70:7241–7246.

Conrad SF, Byeon IJ, DiGeorge AM, Lairmore MD, Tsai MD, Kaumaya PT. 1995. Immunogenicity and conformational properties of an N-linked glycosylated peptide epitope of human T-lymphotropic virus type 1 (HTLV-I). Biomed Pept Proteins Nucleic Acids 1:83–92.

de Thé G, Bomford R. 1993. An HTLV-I vaccine: Why, how, for whom? AIDS Res Hum Retroviruses 9:381–386.

Delebecque F, Combredet C, Gabet AS, Wattel E, Brahic M, Tangy F. 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.

Derse D, Mikovits J, Polianova M, Felber BK, Ruscetti F. 1995. Virions released from cells transfected with a molecular clone of human T-cell leukemia virus type I give rise to primary and secondary infections of T cells. J Virol 69:1907–1912.

Dewan MZ, Terashima K, Taruishi M, Hasegawa H, Ito M, Tanaka Y, Mori N, Sata T, Koyanagi Y, Maeda M, Kubuki Y, Okayama A, Fujii M, Yamamoto N. 2003. Rapid tumor formation of human T-cell leukemia virus type 1-infected cell lines in novel NOD-SCID/gammac(null) mice: Suppression by an inhibitor against NF-kappaB. J Virol 77:5286–5294.

El Hajj H, Khalil B, Ghandour B, Nasr R, Shahine S, Ghantous A, Abdel-Samad R, Sinjab A, Hasegawa H, Jabbour M, Hall WW, Zaatari G, Dbaibo G, Pisano C, Bazarbachi A, Darwiche N. 2014. Preclinical efficacy of the synthetic retinoid ST1926 for treating adult T-cell leukemia/lymphoma. Blood 124: 2072–2080.

Fang J, Kushida S, Feng R, Tanaka M, Kawamura T, Abe H, Maeda N, Onobori M, Hori M, Uchida K, Miwa M. 1998. Transmission of human T-cell leukemia virus type 1 to mice. J Virol 72:3952–3957.

Feuer G, Stewart SA, Baird SM, Lee F, Feuer R, Chen IS. 1995. Potential role of natural killer cells in controlling tumorigenesis by human T-cell leukemia viruses. J Virol 69:1328–1333.

Feuer G, Zack JA, Harrington WJ Jr., Valderama R, Rosenblatt JD, Wachsman W, Baird SM, Chen IS. 1993. Establishment of human T-cell leukemia virus type I T-cell lymphomas in severe combined immunodeficient mice. Blood 82:722–731.

Florins A, Boxus M, Vandermeers F, Verlaeten O, Bouzar AB, Defoiche J, Hubaux R, Burny A, Kettmann R, Willems L. 2008. Emphasis on cell turnover in two hosts infected by bovine leukemia virus: A rationale for host susceptibility to disease. Vet Immunol Immunopathol 125:1–7.

Frangione-Beebe M, Albrecht B, Dakappagari N, Rose RT, Brooks CL, Schwendeman SP, Lairmore MD, Kaumaya PT. 2000. Enhanced immunogenicity of a conformational epitope of human T-lymphotropic virus type 1 using a novel chimeric peptide. Vaccine 19:1068–1081.

Gao L, Deng H, Zhao H, Hirbe A, Harding J, Ratner L, Weilbaecher K. 2005. HTLV-1 Tax transgenic mice develop spontaneous osteolytic bone metastases prevented by osteoclast inhibition. Blood 106:4294–4302.

Gessain A, Cassar O. 2012. Epidemiological aspects and world distribution of HTLV-1 infection. Front Microbiol 3:388.

Grassmann R, Aboud M, Jeang KT. 2005. Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene 24: 5976–5985.

Green JE, Hinrichs SH, Vogel J, Jay G. 1989. Exocrinopathy resembling Sjogren's syndrome in HTLV-1 tax transgenic mice. Nature 341:72–74.

Grossman WJ, Kimata JT, Wong FH, Zutter M, Ley TJ, Ratner L. 1995. Development of leukemia in mice transgenic for the tax gene of human T-cell leukemia virus type I. Proc Natl Acad Sci USA 92:1057–1061.

Habu K, Nakayama-Yamada J, Asano M, Saijo S, Itagaki K, Horai R, Yamamoto H, Sekiguchi T, Nosaka T, Hatanaka M, Iwakura Y. 1999. The human T cell leukemia virus type I-tax gene is responsible for the development of both inflammatory

polyarthropathy resembling rheumatoid arthritis and noninflammatory ankylotic arthropathy in transgenic mice. J Immunol 162:2956–2963.

Hanabuchi S, Ohashi T, Koya Y, Kato H, Hasegawa A, Takemura F, Masuda T, Kannagi M. 2001. Regression of human T-cell leukemia virus type I (HTLV-I)-associated lymphomas in a rat model: Peptide-induced T-cell immunity. J Natl Cancer Inst 93:1775–1783.

Hasegawa H, Sawa H, Lewis MJ, Orba Y, Sheehy N, Yamamoto Y, Ichinohe T, Tsunetsugu-Yokota Y, Katano H, Takahashi H, Matsuda J, Sata T, Kurata T, Nagashima K, Hall WW. 2006. Thymus-derived leukemia-lymphoma in mice transgenic for the Tax gene of human T-lymphotropic virus type I. Nat Med 12:466–472.

Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G, Jay G. 1987. A transgenic mouse model for human neurofibromatosis. Science 237:1340–1343.

Hiraragi H, Kim SJ, Phipps AJ, Silic-Benussi M, Ciminale V, Ratner L, Green PL, Lairmore MD. 2006. Human T-lymphotropic virus type 1 mitochondrion-localizing protein p13(II) is required for viral infectivity in vivo. J Virol 80:3469–3476.

Hirose S, Kotani S, Uemura Y, Fujishita M, Taguchi H, Ohtsuki Y, Miyoshi I. 1988. Milk-borne transmission of human T-cell leukemia virus type I in rabbits. Virology 162:487–489.

Ikebe E, Kawaguchi A, Tezuka K, Taguchi S, Hirose S, Matsumoto T, Mitsui T, Senba K, Nishizono A, Hori M, Hasegawa H, Yamada Y, Ueno T, Tanaka Y, Sawa H, Hall W, Minami Y, Jeang KT, Ogata M, Morishita K, Hasegawa H, Fujisawa J, Iha H. 2013. Oral administration of an HSP90 inhibitor, 17-DMAG, intervenes tumor-cell infiltration into multiple organs and improves survival period for ATL model mice. Blood Cancer J 3:e132.

Imada K, Takaori-Kondo A, Akagi T, Shimotohno K, Sugamura K, Hattori T, Yamabe H, Okuma M, Uchiyama T. 1995. Tumorigenicity of human T-cell leukemia virus type I-infected cell lines in severe combined immunodeficient mice and characterization of the cells proliferating in vivo. Blood 86:2350–2357.

Ishihara S, Tachibana N, Okayama A, Murai K, Tsuda K, Mueller N. 1992. Successful graft of HTLV-I-transformed human T-cells (MT-2) in severe combined immunodeficiency mice treated with anti-asialo GM-1 antibody. Jpn J Cancer Res 83:320–323.

Ishitsuka K, Kunami N, Katsuya H, Nogami R, Ishikawa C, Yotsumoto F, Tanji H, Mori N, Takeshita M, Miyamoto S, Tamura K. 2012. Targeting Bcl-2 family proteins in adult T-cell leukemia/lymphoma: in vitro and in vivo effects of the novel Bcl-2 family inhibitor ABT-737. Cancer Lett 317: 218–225.

Ishitsuka K, Tamura K. 2014. Human T-cell leukaemia virus type I and adult T-cell leukaemia-lymphoma. Lancet Oncol 15: e517–e526.

Iwahara Y, Takehara N, Kataoka R, Sawada T, Ohtsuki Y, Nakachi H, Maehama T, Okayama T, Miyoshi I. 1990. Transmission of HTLV-I to rabbits via semen and breast milk from seropositive healthy persons. Int J Cancer 45:980–983.

Iwakura Y, Tosu M, Yoshida E, Takiguchi M, Sato K, Kitajima I, Nishioka K, Yamamoto K, Takeda T, Hatanaka M, Yamamoto H, Sekiguchi T. 1991. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. Science 253:1026–1028.

Ju W, Zhang M, Petrus M, Maeda M, Pise-Masison CA, Waldmann TA. 2014. Combination of 9-aminoacridine with Campath-1H provides effective therapy for a murine model of adult T-cell leukemia. Retrovirology 11:43.

Kannagi M, Harashima N, Kurihara K, Ohashi T, Utsunomiya A, Tanosaki R, Masuda M, Tomonaga M, Okamura J. 2005. Tumor immunity against adult T-cell leukemia. Cancer Sci 96:249–255.

Karube K, Ohshima K, Tsuchiya T, Yamaguchi T, Kawano R, Suzumiya J, Utsunomiya A, Harada M, Kikuchi M. 2004. Expression of FoxP3, a key molecule in CD4CD25 regulatory T cells, in adult T-cell leukaemia/lymphoma cells. Br J Haematol 126:81–84.

Kataoka R, Takehara N, Iwahara Y, Sawada T, Ohtsuki Y, Dawei Y, Hoshino H, Miyoshi I. 1990. Transmission of HTLV-I by blood transfusion and its prevention by passive immunization in rabbits. Blood 76:1657–1661.

Kimata JT, Wong FH, Wang JJ, Ratner L. 1994. Construction and characterization of infectious human T-cell leukemia virus type 1 molecular clones. Virology 204:656–664.

Koller BH, Smithies O. 1989. Inactivating the beta 2-microglobulin locus in mouse embryonic stem cells by homologous recombination. Proc Natl Acad Sci USA 86:8932–8935.

Kondo A, Imada K, Hattori T, Yamabe H, Tanaka T, Miyasaka M, Okuma M, Uchiyama T. 1993. A model of in vivo cell proliferation of adult T-cell leukemia. Blood 82:2501–2509.

Kotani S, Yoshimoto S, Yamato K, Fujishita M, Yamashita M, Ohtsuki Y, Taguchi H, Miyoshi I. 1986. Serial transmission of human T-cell leukemia virus type I by blood transfusion in rabbits and its prevention by use of X-irradiated stored blood. Int J Cancer 37:843–847.

Kushida S, Maeda N, Fang J, Uchida K, Miwa M. 1997. Establishment of HTLV-1 carrier mice by injection with HTLV-1-producing T cells. Leukemia 11 Suppl 3:260–262.

Kushida S, Matsumura M, Tanaka H, Ami Y, Hori M, Kobayashi M, Uchida K, Yagami K, Kameyama T, Yoshizawa T, Mizusawa H, Iwasaki Y, Miwa M. 1993. HTLV-1-associated myelopathy/tropical spastic paraparesis-like rats by intravenous injection of HTLV-1-producing rabbit or human T-cell line into adult WKA rats. Jpn J Cancer Res 84:831–833.

Kushida S, Mizusawa H, Matsumura M, Tanaka H, Ami Y, Hori M, Yagami K, Kameyama T, Tanaka Y, Yoshida A, Hiroshi N, Kunitada S, Yuzo I, Kazuhiki U, Masanao M. 1994. High incidence of HAM/TSP-like symptoms in WKA rats after administration of human T-cell leukemia virus type 1-producing cells. J Virol 68:7221–7226.

Lal RB, Rudolph DL, Palker TJ, Coligan JE, Folks TM. 1991. A synthetic peptide elicits antibodies reactive with the external glycoprotein of human T cell lymphotropic virus type I. J Gen Virol 72 (Pt 9):2321–2324.

Lapin BA, Yakovleva LA. 2014. Spontaneous and experimental malignancies in non-human primates. J Med Primatol 43:100–110.

Liu Y, Dole K, Stanley JR, Richard V, Rosol TJ, Ratner L, Lairmore M, Feuer G. 2002. Engraftment and tumorigenesis of HTLV-1 transformed T cell lines in SCID/bg and NOD/SCID mice. Leuk Res 26:561–567.

Masamoto I, Yoshimitsu M, Kuroki A, Horai S, Ezinne CC, Kozako T, Hachiman M, Kamada Y, Baba M, Arima N. 2015. Clinical significance of CD70 expression on T cells in human T-lymphotropic virus type-1 carriers and adult T cell leukemia/ lymphoma patients. Leuk Lymphoma 56:1–7.

Matsuoka M, Jeang KT. 2007. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. Nat Rev Cancer 7:270–280.

Mesnard JM, Barbeau B, Devaux C. 2006. HBZ, a new important player in the mystery of adult T-cell leukemia. Blood 108:3979–3982.

Mitagami Y, Yasunaga J, Kinosada H, Ohshima K, Matsuoka M. 2015. Interferon-gamma promotes inflammation and development of T-cell lymphoma in HTLV-1 bZIP factor transgenic mice. PLoS Pathog 11:e1005120.

Miyazato P, Yasunaga J, Taniguchi Y, Koyanagi Y, Mitsuya H, Matsuoka M. 2006. De novo human T-cell leukemia virus type 1 infection of human lymphocytes in NOD-SCID, common gamma-chain knockout mice. J Virol 80:10683–10691.

Miyoshi I, Yoshimoto S, Kubonishi I, Fujishita M, Ohtsuki Y, Yamashita M, Yamato K, Hirose S, Taguchi H, Niiya K. 1985. Infectious transmission of human T-cell leukemia virus to rabbits. Int J Cancer 35:81–85.

Mizusawa H, Kushida S, Matsamura M, Tanaka H, Ami Y, Hori M, Kobayashi M, Uchida K, Yagami K, Yoshizawa T, Kameyama T, Iwasaki Y, Miwa M. 1994. A neuropathological study of paraparetic rats injected with HTLV-I-producing T cells. J Neurol Sci 126:101–108.

Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G, Jay G. 1987. The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science 237: 1324–1329.

Nitta T, Tanaka M, Sun B, Sugihara E, Kimura M, Kamada Y, Takahashi H, Hanai S, Jiang SW, Fujisawa J, Miwa M. 2008. Reduction of human T-cell leukemia virus type-1 infection in mice lacking nuclear factor-kappaB-inducing kinase. Cancer Sci 99:872–878.

Nomura M, Ohashi T, Nishikawa K, Nishitsuji H, Kurihara K, Hasegawa A, Furuta RA, Fujisawa J, Tanaka Y, Hanabuchi S, Harashima N, Masuda T, Kannagi M. 2004. Repression of tax expression is associated both with resistance of human T-cell leukemia virus type 1-infected T cells to killing by tax-specific cytotoxic T lymphocytes and with impaired tumorigenicity in a rat model. J Virol 78:3827–3836.

Ohbo K, Suda T, Hashiyama M, Mantani A, Ikebe M, Miyakawa K, Moriyama M, Nakamura M, Katsuki M, Takahashi K, Yamamura K, Sugamura K. 1996. Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor gamma chain. Blood 87:956–967.

Ohsugi T, Ishibashi K, Shingu M, Nomura T. 1994. Engraftment of HTLV-I-transformed human T-cell line into SCID mice with NK cell function. J Vet Med Sci 56:601–603.

Ohsugi T, Kumasaka T, Okada S, Ishida T, Yamaguchi K, Horie R, Watanabe T, Umezawa K. 2007. Dehydroxymethylepoxyquinomicin (DHMEQ) therapy reduces tumor formation in mice inoculated with tax-deficient adult T-cell leukemia-derived cell lines. Cancer Lett 257:206–215.

Ohsugi T, Kumasaka T, Okada S, Urano T. 2007. The Tax protein of HTLV-1 promotes oncogenesis in not only immature T cells but also mature T cells. Nat Med 13:527–528.

Ohsugi T, Wakamiya M, Morikawa S, Matsuura K, Kumar JM, Kumasaka T, Yamaguchi K. 2013. Invasion of histiocytic sarcoma into the spinal cord of HTLV-1 tax transgenic mice with HTLV-1-associated myelopathy/tropical spastic paraparesis-like disease. Oncol Res 20:403–410.

Ohsugi T, Yamaguchi K, Kumasaka T, Ishida T, Horie R, Watanabe T, Sakio N, Fujimoto T, Sakamoto N, Urano T. 2004. Rapid tumor death model for evaluation of new therapeutic agents for adult T-cell leukemia. Lab Invest 84:263–266.

Panfil AR, Al-Saleem JJ, Green PL. 2013. Animal models utilized in HTLV-1 research. Virology (Auckl) 4:49–59.

Parrula C, Fernandez SA, Zimmerman B, Lairmore M, Niewiesk S. 2011. Measles virotherapy in a mouse model of adult T-cell leukaemia/lymphoma. J Gen Virol 92:1458–1466.

Parrula C, Zimmerman B, Nadella P, Shu S, Rosol T, Fernandez S, Lairmore M, Niewiesk S. 2009. Expression of tumor invasion factors determines systemic engraftment and induction of humoral hypercalcemia in a mouse model of adult T-cell leukemia. Vet Pathol 46:1003–1014.

Parrula M, Fernandez SA, Landes K, Huey D, Lairmore M, Niewiesk S. 2014. Success of measles virotherapy in ATL depends on type I interferon secretion and responsiveness. Virus Res 189:206–213.

Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL. 2005. Global epidemiology of HTLV-I infection and associated diseases. Oncogene 24:6058–6068.

Rahman S, Manuel SL, Khan ZK, Wigdahl B, Acheampong E, Tangy F, Jain P. 2010. 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. J Immunol 184:5553–5561.

Rauch D, Gross S, Harding J, Niewiesk S, Lairmore M, Piwnica-Worms D, Ratner L. 2009. Imaging spontaneous tumorigenesis: Inflammation precedes development of peripheral NK tumors. Blood 113:1493–1500.

Rauch DA, Harding JC, Ratner L. 2014. IL-15 deficient tax mice reveal a role for IL-1alpha in tumor immunity. PLoS One 9: e85028.

Richard V, Lairmore MD, Green PL, Feuer G, Erbe RS, Albrecht B, D'Souza C, Keller ET, Dai J, Rosol TJ. 2001. Humoral hypercalcemia of malignancy: severe combined immunodeficient/beige mouse model of adult T-cell lymphoma independent of human T-cell lymphotropic virus type-1 tax expression. Am J Pathol 158:2219–2229.

Robek MD, Wong FH, Ratner L. 1998. Human T-cell leukemia virus type 1 pX-I and pX-II open reading frames are dispensable for the immortalization of primary lymphocytes. J Virol 72:4458–4462.

Roncador G, Garcia JF, Maestre L, Lucas E, Menarguez J, Ohshima K, Nakamura S, Banham AH, Piris MA. 2005. FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. Leukemia 19:2247–2253.

Ruddle NH, Li CB, Horne WC, Santiago P, Troiano N, Jay G, Horowitz M, Baron R. 1993. Mice transgenic for HTLV-I LTR-tax exhibit tax expression in bone, skeletal alterations, and high bone turnover. Virology 197:196–204.

Saggioro D, Rosato A, Esposito G, Rosenberg MP, Harrison J, Felber BK, Pavlakis GN, Chieco-Bianchi L. 1997. Inflammatory polyarthropathy and bone remodeling in HTLV-I Tax-transgenic mice. J Acquir Immune Defic Syndr Hum Retrovirol 14:272–280.

Saito M, Tanaka R, Fujii H, Kodama A, Takahashi Y, Matsuzaki T, Takashima H, Tanaka Y. 2014. The neutralizing function of the anti-HTLV-1 antibody is essential in preventing in vivo transmission of HTLV-1 to human T cells in NOD-SCID/gammacnull (NOG) mice. Retrovirology 11:74.

Satou Y, Yasunaga J, Zhao T, Yoshida M, Miyazato P, Takai K, Shimizu K, Ohshima K, Green PL, Ohkura N, et al. 2011. HTLV-1 bZIP factor induces T-cell lymphoma and systemic inflammation in vivo. PLoS Pathog 7:e1001274.

Shimoyama M. 1991. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. A report from the Lymphoma Study Group (1984–1987). Br J Haematol 79:428–437.

Shu ST, Nadella MV, Dirksen WP, Fernandez SA, Thudi NK, Werbeck JL, Lairmore MD, Rosol TJ. 2007. A novel bioluminescent mouse model and effective therapy for adult T-cell leukemia/lymphoma. Cancer Res 67:11859–11866.

Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, McKenna S, Mobraaten L, Rajan TV, Greiner DL. 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol 154:180–191.

Silverman LR, Phipps AJ, Montgomery A, Fernandez S, Tsukahara T, Ratner L, Lairmore MD. 2005. In vivo analysis of replication and immunogenicity of proviral clones of human T-lymphotropic virus type 1 with selective envelope surface-unit mutations. Blood 106:3602–3608.

Silverman LR, Phipps AJ, Montgomery A, Ratner L, Lairmore MD. 2004. Human T-lymphotropic virus type 1 open reading frame II encoded p30II is required for in vivo replication: evidence of in vivo reversion. J Virol 78:3837–3845.

Sugata K, Satou Y, Yasunaga J, Hara H, Ohshima K, Utsunomiya A, Mitsuyama M, Matsuoka M. 2012. HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines. Blood 119:434–444.

Sun B, Fang J, Yagami K, Kushida S, Tanaka M, Uchida K, Miwa M. 1999. Age-dependent paraparesis in WKA rats: Evaluation of MHC k-haplotype and HTLV-1 infection. J Neurol Sci 167:16–21.

Takaori-Kondo A, Imada K, Yamamoto I, Kunitomi A, Numata Y, Sawada H, Uchiyama T. 1998. Parathyroid hormone-related protein-induced hypercalcemia in SCID mice engrafted with adult T-cell leukemia cells. Blood 91:4747–4751.

Tanaka M, Sun B, Fang J, Nitta T, Yoshida T, Kohtoh S, Kikukawa H, Hanai S, Uchida K, Miwa M. 2001. Human T-cell leukemia virus type 1 (HTLV-1) infection of mice: Proliferation of cell clones with integrated HTLV-1 provirus in lymphoid organs. J Virol 75:4420–4423.

Tanaka Y, Tanaka R, Terada E, Koyanagi Y, Miyano-Kurosaki N, Yamamoto N, Baba E, Nakamura M, Shida H. 1994. Induction of antibody responses that neutralize human T-cell leukemia virus type I infection in vitro and in vivo by peptide immunization. J Virol 68:6323–6331.

Tanaka Y, Zeng L, Shiraki H, Shida H, Tozawa H. 1991. Identification of a neutralization epitope on the envelope gp46 antigen of human T cell leukemia virus type I and induction of neutralizing antibody by peptide immunization. J Immunol 147:354–360.

Tezuka K, Xun R, Tei M, Ueno T, Tanaka M, Takenouchi N, Fujisawa J. 2014. An animal model of adult T-cell leukemia: Humanized mice with HTLV-1-specific immunity. Blood 123:346–355.

Tobinai K. 2009. Current management of adult T-cell leukemia/lymphoma. Oncology (Williston Park) 23:1250–1256.

Tsukasaki K, Tobinai K. 2014. Human T-cell lymphotropic virus type I-associated adult T-cell leukemia-lymphoma: New directions in clinical research. Clin Cancer Res 20:5217–5225.

Uchiyama T. 1996. ATL and HTLV-I: In vivo cell growth of ATL cells. J Clin Immunol 16:305–314.

Uemura Y, Kotani S, Yoshimoto S, Fujishita M, Yamashita M, Ohtsuki Y, Taguchi H, Miyoshi I. 1987. Mother-to-offspring transmission of human T cell leukemia virus type I in rabbits. Blood 69:1255–1258.

Uemura Y, Kotani S, Yoshimoto S, Fujishita M, Yano S, Ohtsuki Y, Miyoshi I. 1986. Oral transmission of human T-cell leukemia virus type I in the rabbit. Jpn J Cancer Res 77:970–973.

Villaudy J, Wencker M, Gadot N, Gillet NA, Scoazec JY, Gazzolo L, Manz MG, Bangham CR, Dodon MD. 2011. HTLV-1 propels thymic human T cell development in "human immune system" Rag2(-)/(-) gamma c(-)/(-) mice. PLoS Pathog 7:e1002231.

Watters KM, Dean J, Hasegawa H, Sawa H, Hall W, Sheehy N. 2010. Cytokine and growth factor expression by HTLV-1 Lck-tax transgenic cells in SCID mice. AIDS Res Hum Retroviruses 26:593–603.

Yamazaki J, Mizukami T, Takizawa K, Kuramitsu M, Momose H, Masumi A, Ami Y, Hasegawa H, Hall WW, Tsujimoto H, Hamaguchi I, Yamaguchi K. 2009. Identification of cancer stem cells in a Tax-transgenic (Tax-Tg) mouse model of adult T-cell leukemia/lymphoma. Blood 114:2709–2720.

Yoshie O, Fujisawa R, Nakayama T, Harasawa H, Tago H, Izawa D, Hieshima K, Tatsumi Y, Matsushima K, Hasegawa H, Kanamaru A, Kamihira S, Yamada Y. 2002. Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. Blood 99:1505–1511.

Zhang Z, Zhang M, Ravetch JV, Goldman C, Waldmann TA. 2003. Effective therapy for a murine model of adult T-cell leukemia with the humanized anti-CD2 monoclonal antibody, MEDI-507. Blood 102:284–288.

Zhao T, Satou Y, Matsuoka M. 2014. Development of T cell lymphoma in HTLV-1 bZIP factor and Tax double transgenic mice. Arch Virol 159:1849–1856.

Zhao TM, Robinson MA, Bowers FS, Kindt TJ. 1995. Characterization of an infectious molecular clone of human T-cell leukemia virus type I. J Virol 69:2024–2030.

Zimmerman B, Sargeant A, Landes K, Fernandez SA, Chen CS, Lairmore MD. 2011. Efficacy of novel histone deacetylase inhibitor, AR42, in a mouse model of, human T-lymphotropic virus type 1 adult T cell lymphoma. Leuk Res 35:1491–1497.