Animal models for human T-lymphotropic virus type 1 (HTLV-1) infection and transformation

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Abstract

Over the past 25 years, animal models of human T-lymphotropic virus type 1 (HTLV-1) infection and transformation have provided critical knowledge about viral and host factors in adult T-cell leukemia/lymphoma (ATL). The virus consistently infects rabbits, some non-human primates, and to a lesser extent rats. In addition to providing fundamental concepts in viral transmission and immune responses against HTLV-1 infection, these models have provided new information about the role of viral proteins in carcinogenesis. Mice and rats, in particular immunodeficient strains, are useful models to assess immunologic parameters mediating tumor outgrowth and therapeutic invention strategies against lymphoma. Genetically altered mice including both transgenic and knockout mice offer important models to test the role of specific viral and host genes in the development of HTLV-1-associated lymphoma. Novel approaches in genetic manipulation of both HTLV-1 and animal models are available to address the complex questions that remain about viral-mediated mechanisms of cell transformation and disease. Current progress in the understanding of the molecular events of HTLV-1 infection and transformation suggests that answers to these questions are approachable using animal models of HTLV-1-associated lymphoma

Keywords

HTLV-1; animal models; leukemia; lymphoma

Introduction

Human T-lymphtropic virus type 1 (HTLV-1), a member of the deltaretroviruses, is the causative agent of adult T-cell leukemia/lymphoma (ATL) (Yoshida *et al*., 1982), a highly aggressive CD4+ T-cell malignancy. Over the past 25 years, a variety of animal models of HTLV-1, infection and transformation have provided fundamental information about viral and

host determinants of this devastating malignancy (Table 1). The virus consistently infects rabbits (Akagi *et al*., 1985; Lairmore *et al*., 1992), some non-human primates (Nakamura *et al*., 1987; Murata *et al*., 1996), and to a lesser extent rats (Suga *et al*., 1991; Ibrahim *et al*., 1994). Viral transmission in mice using typical methods of infection produces inconsistent infections and limited virus expression in tissues (Fang *et al*., 1998; Feng *et al*., 2001; Furuta *et al*., 2002a, b; Nitta *et al*., 2003). Non-human primates have been infected with HTLV-1 and certain species have a natural infection with simian T-lymphotropic virus infection type 1 (STLV-1) (Gessain and Dethe 1996; Takemura *et al*., 2002; Gabet *et al*., 2003). The squirrel monkey has been successfully infected with HTLV-1 and offers an attractive non-human primate model of HTLV-1 for vaccine testing (Kazanji, 2000; Kazanji *et al*., 2001; Mortreux *et al*., 2001a; Sundaram *et al*., 2004). Rats have been infected with HTLV-1-producing cells and offer a tractable model of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/ATL), the neurologic disease associated with the viral infection (Suga *et al*., 1991; Ishiguro *et al*., 1992; Kasai *et al*., 1999; Sun *et al*., 1999; Kannagi *et al*., 2000; Hakata *et al*., 2001). In addition, rats have been used to test the role of cell-mediated immunity to the infection (Kannagi *et al*., 2000; Hasegawa *et al*., 2003). However, controversy exists regarding the reproducibility of the viral infection in rats (Ibrahim *et al*., 1994). Bovine leukemia virus (BLV) infection of sheep offers a reliable model of disease associated with deltaretrovirus infections and insight into viral genetic determinants of tumor induction (Willems *et al*., 2000). This review will focus on current progress in the development of animal models of HTLV-1 infection and transformation.

HTLV-1 replication and mechanisms of carcinogenesis

Understanding HTLV-1 replication forms the basis of development for animal models of the viral-induced malignancy. HTLV-1 is a complex retrovirus with type C retroviral morphology. The virus belongs to the deltaretrovirus group along with BLV and other primate lymphotropic viruses including STLV-1. Unlike simple retroviruses, complex retroviruses like HTLV-1 carry additional genes to encode for several regulatory and accessory proteins. As a complex retrovirus, HTLV-1 encodes regulatory proteins from the pX region (between *env* and 3′-LTR), by alternative splicing from four open reading frames (ORF). ORF IV and ORF III encode regulatory proteins Tax (Transcriptional Activator of pX region) and Rex, respectively (Kiyokawa *et al*., 1985; Seiki *et al*., 1985). Tax, a 40 kDa nuclear protein produced from a doubly spliced mRNA, is transactivator of HTLV-1 gene transcription from the viral LTR (Felber *et al*., 1985; Sodroski *et al*., 1985). Tax forms complexes with DNA-binding proteins, such as CREB, to contact GC-rich sequences flanking the Tax-responsive element 1 (TRE-1) for the transactivation of the LTR (Kimzey and Dynan, 1999; Lenzmeier *et al*., 1999). Tax enhances the binding of cyclic AMP response/activator of transcription proteins to the TRE-1 and several basic leucine zipper proteins to the TRE-2 (Perini *et al*., 1995). In addition, Tax regulates the expression of numerous cellular genes, predominantly by induction of the transcription factors nuclear factor kappa of B cells (NF-κB) and serum response factor, independent of CREB activation (Gatza *et al*., 2003; Jeang *et al*., 2004; Kehn *et al*., 2004). Rex, a nucleolar localizing 27 kDa protein, is responsible for nuclear export of unspliced (*gag/pol*) and singly spliced (*env*) viral RNA to the cytoplasm. Even though Rex is not required for *in vitro* immortalization by HTLV-1, Ye *et al*. (2003) reported that Rex is critical for efficient infection of cells and persistence *in vivo*.

In addition to the regulatory proteins Tax and Rex, the HTLV-1 pX genome region encodes four accessory proteins, p12[I], p27[I], p13[II], and p30[II] from alternatively spliced forms of mRNA in ORF I and II (Berneman *et al*., 1992; Koralnik *et al*., 1992, 1993; Ciminale *et al*., 1995). These proteins are important for *in vivo* viral infectivity, host cell activation, and regulation of gene transcription (Collins *et al*., 1998; Ciminale *et al*., 1999; Trovato *et al*., 1999; Albrecht *et al*., 2000, 2002; Bartoe *et al*., 2000; D'Agostino *et al*., 2000; Zhang *et al*., 2000, 2001; Ding

et al., 2002; Franchini *et al.*, 2003; Kim *et al.*, 2003; Michael *et al.*, 2004; Nicot *et al.*, 2004; Younis *et al.*, 2004). The role of these accessory proteins in HTLV-1-mediated transformation has not been elucidated, but they are clearly important in the ability of the virus to infect and spread *in vivo*.

Adult T-cell leukemia/lymphoma

Animal models of HTLV-1 transformation ideally mimic the variety of forms of ATL- or HTLV-1-associated diseases. Typically, 1–5% of HTLV-1-infected individuals develop ATL after a latent period of 20–30 years (Yamaguchi and Takatsuki, 1993; Cleghorn *et al.*, 1995). ATL is an aggressive T-cell malignancy with a leukemic phase characterized by circulating, activated CD4+/CD25+ T cells (Uchiyama *et al.*, 1977). Infection early in life is associated with the development of ATL and the estimated lifetime risk is about 5% in individuals infected before the age of 20 years (Cleghorn *et al.*, 1995; Wilks *et al.*, 1996). The incidence rate is 2–4 per 100 000 person-years and males have higher risk than females (Kondo *et al.*, 1989; Cleghorn *et al.*, 1995). The clinical features, treatment and prevention of ATL are reviewed in this volume.

The development of ATL has been the focus of many investigations, but the exact mechanism is not completely understood. After infecting CD4+CD25+ T cells, the HTLV-1 provirus is randomly integrated into the host genome, where it persists for years. Although HTLV-1 replicates via reverse transcription during the early stages of infection, the virus genome is effectively replicated during clonal expansion of infected cells (Mortreux *et al.*, 2003). Typically, there is a progression from polyclonal to oligoclonal and then to monoclonal proliferation *in vivo*, which is achieved while the cells become interleukin 2 (IL-2) independent (Yoshida *et al.*, 1984; Hollsberg *et al.*, 1992; Franchini, 1995). Based on the long clinical latency and the low percentage of individuals who develop ATL, T-cell transformation is believed to be the result of a series of cellular alterations and/or mutations. Transformation of infected lymphocytes is believed to be initiated through induction of cellular genes and alterations in critical cellular activation and death pathways by the viral transactivator Tax (Gatza *et al.*, 2003).

Intriguingly, despite a strong immune response mounted against the HTLV-1, the virus is able to persist in the host. Several possible mechanisms have been suggested to explain this observation:often the genome is partly deleted, resulting in defective virus that may provide a mechanism for escape from immune surveillance (Bangham 2000, 2003a, Bangham b). Recent reports (Asquith *et al.*, 2000) suggest that HTLV-1 infection is in a dynamic balance between the immune response and virus replication (Asquith *et al.*, 2000). What determines whether an HTLV-1-infected individual will progress to develop ATL is unclear. In the early course of HTLV-1 infection, integration of the provirus into host cell chromatin is random and polyclonal (Mortreux *et al.*, 2003). During the long premalignant phase, there is oligoclonal expansion of HTLV-1-infected cells (Wattel *et al.*, 1995; Cavrois *et al.*, 1996; Etoh *et al.*, 1997). Somatic mutations are increased in peripheral blood mononuclear cell (PBMC) DNA from ATL patients compared to asymptomatic carriers (Mortreux *et al.*, 2003). Somatic mutations are increased in both the provirus and within the proviral flanking sequences, and these mutations are thought to primarily arise during the clonal proliferative phase rather than during reverse transcription (Mortreux *et al.*, 2001b).

Factors that impair the cellular immune response and promote T-cell proliferation are thought to increase genetic instability of the virus and therefore increase the likelihood of development of ATL (Mortreux *et al.*, 2003). One such factor is the HTLV-1 Tax protein. Tax expression promotes cellular proliferation and increase genetic instability (reviewed in Yoshida, 2001; Mortreux *et al.*, 2003). In general, one of the tenets of malignant progression is that it requires mutations in several genes, including oncogenes, tumor suppressor genes, DNA repair genes,

and apoptosis-regulating genes. Tax has been demonstrated to regulate gene expression of the apoptosis-regulating genes, *bcl-xL* (Tsukahara *et al*., 1999) and *bax* (Brauweiler *et al*., 1997), the DNA repair genes, *PCNA* (Ressler *et al*., 1997) and *β-polymerase* (Jeang *et al*., 1990), the cell cycle regulators, *cyclin D2*, *cyclin E*, *E2F1*, *CDK2*, *CDK4*, *CDK6*, *p19 (INK4d)*, and *p27 (Kip1)* (Iwanaga *et al*., 2001), and the tumor suppressor, p53 (de La *et al*., 2003). Therefore, Tax can promote malignant progression not only by increasing cellular proliferation but also by altering the expression of genes known to have the potential to contribute to malignant progression. Thus, over the past two decades, most animal models of ATL have focused on Tax and its role in lymphocyte transformation.

The route of primary HTLV-1 infection is also correlated with the course of clinical disease. Specially, most cases of ATL occur subsequent to mucosal exposure, whereas most cases of HAM/TSP occur subsequent to intravenous exposure (Osame *et al*., 1990; Kannagi *et al*., 2000). Interestingly, oral inoculation of rats with HTLV-1 generally results in a persistent infection with immune unresponsiveness (Kato *et al*., 1998), whereas intravenous inoculation results in strong antibody and T-cell responses (Kannagi *et al*., 2000). Extrapolating from this, it is currently believed that intravenous exposure in humans also results in the strong immune response typical of HAM/TSP patients, whereas oral (mucosal) exposure allows for an initial diminished immune response and subsequent survival and outgrowth of infected CD4+ T cells. Intravenous exposure initially leads to infection of a large number of circulating T-lymphocytes, whereas mucosal exposure initially leads to infection of macrophage and dendritic cells and only a small number of T-lymphocytes (reviewed in Grant *et al*., 2002). HTLV-1 has the ability to stimulate T-lymphocytes to enter the cell cycle and promote high levels of gene expression, whereas mucosal dendritic cells and macrophages are postmitotic and therefore not likely to produce high levels of virus following infection (Grant *et al*., 2002). As immune response correlates with virus production, the lower levels of viral production by macrophages and dendritic cells may lead to an initially diminished immune response following oral exposure.

Animal models of HTLV-1 infection and disease

Rabbit models

Rabbits are used extensively as a model of HTLV-1 infection in humans because of the ease and consistency of transmission of the viral infection in this species. Infectivity for rabbits was first demonstrated in the mid-1980s using intravenous inoculations of the MT-2 cell line (Akagi *et al*., 1985), a T-cell leukemia cell line established from a patient with ATL, and with the Ra-1 cell line (Miyoshi *et al*., 1985), a rabbit lymphocyte cell line derived from cocultivation of rabbit lymphocytes with MT-2 cells. Early studies in rabbits verified routes of transmission (e.g. blood, semen, milk) for the virus infection (Kotani *et al*., 1986; Uemura *et al*., 1986, 1987; Hirose *et al*., 1988; Iwahara *et al*., 1990; Kataoka *et al*., 1990). Pioneering studies utilizing the rabbit model of HTLV-1 have provided important clues as to the number of cells capable of transmitting the virus infection (Kataoka *et al*., 1990) and effective means to prevent the transmission of the virus (Takehara *et al*., 1989; Kataoka *et al*., 1990; Sawada *et al*., 1991; Miyoshi *et al*., 1992; Tanaka *et al*., 1994).

The rabbit model has provided important knowledge of the immune response against HTLV-1 infection. Early studies defined the sequential development of antibodies against the virus infection (Cockerell *et al*., 1990), and methods to detect HTLV-1 proviral DNA in infected tissues (Cockerell *et al*., 1990). Inoculation of rabbits with HTLV-1-infected cell lines derived from patients with ATL or HAM/TSP demonstrate the heterogeneity in the biological response to HTLV-1 infection (Lairmore *et al*., 1992). 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). Soon after, it was demonstrated that peptide immunization with amino acids 190–199 of the Env protein could protect rabbits from subsequent HTLV-1 challenge, opening the possibility for vaccine development (Tanaka *et al*., 1994). More complex synthetic peptides, which use chimeric constructs that mimic native viral proteins, have also been generated and tested in rabbit models (Conrad *et al*., 1995; Frangione-Beebe *et al*., 2000).

Infectious molecular clones of HTLV-1 were first developed in the mid-1990s (Kimata *et al*., 1994; Derse *et al*., 1995; Zhao *et al*., 1995). These molecular clones were used to immortalize human PBMCs to create the ACH cell line, which was then used to infect rabbits (Collins *et al*., 1996). It was demonstrated that the lethally irradiated ACH cell line successfully establishes infection in the PBMCs of rabbits (Collins *et al*., 1996). Subsequently, ACH clones with mutations within the ORF encoding the HTLV-1 accessory proteins, p12^{I}, p13^{II}, and p30^{II}, were generated (Robek *et al*., 1998), and inoculated into rabbits to demonstrate the necessity of these accessory proteins for establishment of infection and maintenance of proviral loads (Collins *et al*., 1998; Bartoe *et al*., 2000; Silverman *et al*., 2004). The necessity of the Rex protein for *in vivo* infection has also recently been demonstrated in the rabbit model (Ye *et al*., 2003).

Establishment of a rabbit model of clinical HTLV-1 disease has been more problematic. In the majority of studies, rabbit infection has paralleled the asymptomatic infection of humans. A few groups have reproduced an 'ATL-like disease' via intraperitoneal or intravenous injection of HTLV-1-transformed cells; however, this required a minimum of 1×10^8 cells in the inoculum, and death occurred within the first few weeks of inoculation (Seto *et al*., 1988; Ogawa *et al*., 1989; Zhao *et al*., 1993). These studies did not demonstrate if the leukemic cells were from the inoculum or generated *de novo*. Sporadic reports of clinical disease in HTLV-1-infected rabbits include uveitis (Taguchi *et al*., 1993), cutaneous lymphoma (Simpson *et al*., 1996; Kindt *et al*., 2000), and thymoma (Zhao *et al*., 2002). In each of these cases, clinical disease developed after 1 year and usually several years after the initial infection.

Rat models

Experimental infection of rats with HTLV-1 was first established in 1991 (Suga *et al*., 1991). Although initial experimental infection was achieved with F344 rats, it was later established that there was considerable differences in the response of various rat strains to HTLV-1 infection (Ishiguro *et al*., 1992; Kushida *et al*., 1993; Ibrahim *et al*., 1994). Wistar–King–Aptekman– Hokudai (WKAH) rats emerged as a model of HAM/TSP. HTLV-1-infected WKAH rats develop spastic paraparesis with degenerative thoracic spinal cord and peripheral nerve lesions several months following inoculation (Ishiguro *et al*., 1992; Kushida *et al*., 1993). The pathology of rat HAM/TSP differs from that seen in humans. Lesions in humans have a marked T-cell infiltration of affected regions, whereas lymphocytes are not seen in the lesions in rats (Yoshiki, 1995). Subsequent studies defined the time periods over which the pathologic changes occur (Ohya *et al*., 1997, 2000), and indicated that apoptosis of oligodendrocytes and Schwann cells is the primary event leading to demyelination (Yoshiki 1995, 1997; Ohya *et al*., 1997, 2000). Macrophages are seen in the lesions of rats in response to the demyelination. Production of HTLV-1 pX mRNA, tumor necrosis factor (TNF) alpha mRNA, as well as altered expression of the apoptosis-modifying genes, *bcl-2*, *bax*, and *p53*, have been identified within the lesions (Ohya *et al*., 1997, 2000; Tomaru *et al*., 2003). In addition, HTLV-1 provirus has been identified in microglial cells and macrophages associated with lesions (Kasai *et al*., 1999).

Development of rat models for clinical ATL has required the use of immunodeficient rats. Ohashi *et al*. (1999) demonstrated that an 'ATL-like lymphoproliferative disease' could be established in adult nude (nu/nu) rats following inoculation of some, but not all, HTLV-1-

immortalized cell lines. This led to studies that examined methods of protection against tumor development, including adoptive transfer of T cells (Kannagi *et al*., 2000) and Tax-specific peptide vaccines (Hanabuchi *et al*., 2001). A protective effect was achieved with each of these systems. Most recently, a protective effect against tumor formation in nude rats was achieved with Tax-specific small interfering RNAs (siRNA) (Nomura *et al*., 2004).

Non-human primate models

Experimental HTLV-1 infection, without disease development, in non-human primates was demonstrated in several monkey species inoculated with MT-2 cells, Ra-1 cells, or autologous HTLV-1-infected cell lines (Miyoshi *et al*., 1984; Yamamoto *et al*., 1984; Nakamura *et al*., 1986). Recently, the squirrel monkey has been established as an experimental model of HTLV-1 infection. Peripheral lymphocytes, spleen, and lymph nodes were verified as major reservoirs for HTLV-1 virus during the early phase of infection (Kazanji *et al*., 1997, 2000; Kazanji, 2000). It was subsequently established that similar to humans, HTLV-1 infection in squirrel monkeys begins through reverse transcription of the virus genome, which is then followed by clonal expansion of infected cells (Mortreux *et al*., 2001a).

Early studies tested for antibodies specific for HTLV-1 membrane antigens in macaques with malignant lymphoma (Homma *et al*., 1984; Kanki *et al*., 1985). An experimentally HTLV-1-inoculated rhesus macaque developed arthritis, uveitis, and polymyositis (Beilke *et al*., 1996). More recently, development of clinical disease was reported in pig-tailed macaques following inoculation with a pig-tailed macaque cell line persistently infected with the ACH HTLV-1 molecular clone (McGinn *et al*., 2002). In this report, pig-tailed macaques died naturally at 35–82 weeks postinoculation with lymphopenia, arthropathy, and diarrhea. Like humans with HTLV-1 infection, macques that survived exhibited various combinations of rash, diarrhea, lymphadenopathy, and lymphopenia.

Vaccine candidates against HTLV-1 infection have been tested in non-human primates as early as 1987 (Nakamura *et al*., 1987). Subsequently, successful passive immunization has been achieved with hyper-immune globulin from healthy donors (Murata *et al*., 1996; Akari *et al*., 1997). Successful immunization against HTLV-1 infection has also been accomplished with recombinant vaccinia virus expressing envelope or gag gene products in combination with DNA vaccines (Ibuki *et al*., 1997; Kazanji *et al*., 2001).

Mouse models of HTLV-1 infection

Mouse models of ATL would be useful in providing a small and inexpensive animal for studies of pathogenesis, treatment, and prevention. Unfortunately, HTLV-1 does not efficiently infect murine cells, and thus, mouse models must be manipulated to establish HTLV-1 infection. HTLV-1 carrier mice can be established by intraperitoneal inoculation of MT-2 cells into C3H/HeJ and BALB/c mice (Kushida *et al*., 1997; Fang *et al*., 1998; Feng *et al*., 1999). Intraperitoneal injection of HTLV-1-producing MT-2 cells into neonatal C3H/HeJ mice did not result in detectable antigen or antibody production, although provirus was detected in spleen, lymph node, and thymus at 15 weeks of age and was integrated in the mouse genome. In splenocytes, there was preferential infection of T-lymphocytes. After 18 months, HTLV-1 provirus was detected in spleen DNA in eight of nine C3H/HeJ mice, with polyclonal integration (Tanaka *et al*., 2001). Within these mice, provirus was identified within CD4+ T cells, CD8+ T cells, B cells, and granulocytes within the spleen (Feng *et al*., 1999). Provirus was also identified within various tissues including thymus, lymph nodes, lung, liver, and kidney, although the cell type containing provirus in these organs was not identified, and the proviral loads were not quantitated (Kushida *et al*., 1997; Fang *et al*., 1998). Interestingly, neither viral mRNA production nor an HTLV-1 antibody response was found in the majority of these mice, thus this model fails to mimic persistently infection humans (Kushida *et al*.,

1997; Fang *et al*., 1998). Moreover, similar to the rabbit and rat models, progression to ATL has not been demonstrated in the immunocompetent mouse.

In an attempt to improve the efficiency of infection, a chimeric HTLV-1 virus was used in which the HTLV-1 *env* gene was replaced by the ecotropic Moloney murine leukemia virus *env* gene (Delebecque *et al*., 2002). The chimeric virus was able to replicate in murine cells in culture, but T-cell transformation was not evaluated. Infection of BALB/c, C3H/HeJ, 129Sv, and 129SvIFNAR−/− mice resulted in persistent infection with 500–800 proviral copies/10^5 splenocytes in the majority of mice. In addition, virus was detected in several organs, including lymphoid organs, CNS, lung, and gonads. Importantly, this model demonstrated preferential infection of CD4+ cells and oligoclonal integration of the virus in infected cells. While these mice did not develop lesions, they did have persistent humoral and cellular immune responses.

Tumor transplant models in mice

The severe combined immunodeficiency (SCID) mouse has been a successful model to investigate the proliferative and tumorigenic potential of ATL cells (Ishihara *et al*., 1992; Feuer *et al*., 1993; Kondo *et al*., 1993; Ohsugi *et al*., 1994). SCID mice inoculated with ATL cells succumb to lymphomas, and tumor cells recovered from mice retain the phenotypic and genotypic characteristics of the original tumor cell inoculate (Feuer *et al*., 1993; Kondo *et al*., 1993; Imada *et al*., 1995). Interestingly, HTLV-1-infected cell lines of nonleukemic origin are not tumorigenic following SCID mouse engraftment (Feuer *et al*., 1995; Imada *et al*., 1995; Uchiyama, 1996). Inoculation of HTLV-1-infected nonleukemic cell lines will form tumors in SCID mice only when natural killer (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). Murine NK cells directly mediate cytolysis of cells harboring active HTLV-1 gene expression, suggesting that the absence of viral gene expression in ATL cells contributes to the ability of these cells to evade immune surveillance in humans (Stewart *et al*., 1996). The absence of viral gene expression in the HTLV-1 leukemias of SCID mice has been confirmed (Imada *et al*., 1995, 1996). The inability of HTLV-1-infected nonleukemic cell lines to induce tumorigenesis has also been recently demonstrated in SCID/ bg and nonobese diabetic/SCID (NOD/SCID) mice (Liu *et al*., 2002). Using an SCID/bg mouse model, Richard *et al*. (2001) were able to develop a model for ATL with associated humoral hypercalcemia of malignancy. Interestingly, elevation of parathyroid hormone-related protein in this model was shown to be independent of Tax expression. SCID models of ATL have proven useful in examining treatment strategies for ATL. Variable success in tumor suppression has been achieved with the proteasome inhibitor, PS-341 (Tan and Waldmann, 2002), humanized anti-CD2 monoclonal antibody (Zhang *et al*., 2003b), an NF-*κ*B inhibitor (Dewan *et al*., 2003), and humanized anti-CD52 monoclonal antibody (Zhang *et al*., 2003a). Recently, SCID mice less than 5 weeks old with low NK cell activity developed rapid tumor formation resulting in death (Ohsugi *et al*., 2004). In summary, transplantation models demonstrate unique properties of ATL cells compared to T-lymphocytes immortalized by HTLV-1 in culture, which correlate with limited viral gene expression and resistance to NK cell-mediated cytotoxicity.

Transgenic models

Transgenic mouse models of HTLV-1 have provided an understanding of the role of Tax and Tax-mediated disruption of lymphocyte function or cytokines in HTLV-1-associated lesions. Nerenberg (1990) described neurofibromas in HTLV-1 promoter (LTR)-Tax transgenic mice and used antisense inhibition of NF-*κ*B to demonstrate its role in tumor growth. Lymphocyte-mediated arthropathy developed in Tax transgenic mice controlled through the HTLV-1 promoter (Iwakura *et al*., 1991, 1995; Yamamoto *et al*., 1993; Fujisawa *et al*., 1996). Increased levels of nerve growth factor, granulocyte–macrophage colony-stimulating factor (GM-CSF)

and IL-2 receptor also developed in transgenic mice in which Tax was expressed from the human granzyme B promoter (GzmB) (Grossman and Ratner, 1997). Transgenic mice expressing Tax under the regulatory control of the CD3-epsilon promoter–enhancer manifested salivary and mammary adenomas (Hall *et al.*, 1998). Overall, these studies provide strong support for the oncogenic capabilities of Tax, although they did not demonstrate mechanisms of carcinogenesis.

To improve Tax expression in lymphoid compartments, various investigators have utilized alternative promoters in transgenic mice. HTLV-LTR-c-myc and immunoglobulin enhancer/ promoter (Ig-enh)-Tax transgenic mice lines have been crossed resulting in the development of a variety of tumors (Benvenisty *et al.*, 1992). Transgenic mice that specifically target the mature T-cell compartment by using the GzmB promoter develop large granular lymphocytic (LGL) tumor development (Grossman *et al.*, 1995). Tumors composed of large granular lymphocytes developed in these mice on the tail, legs, and ears. Another interesting feature of the GzmB-Tax transgenic mice is the presence of osteolytic bone lesions, similar to those found in ATL patients (K Weilbaecher, Washington University, manuscript submitted). This represents a unique animal model with a high frequency of metastatic bone lesions, thus providing opportunities to study pathogenesis and treatment of this common complication of metastatic tumors in humans. Lines of LGL cells cultured from these mice displayed surface markers indicating a pre-NK cell lineage (positive for FcγRII/III, IL-2β, CD44, Thy 1.2, 5E6) (Grossman and Ratner, 1997). Tumors arising in these mice exhibited high levels of NF-κB expression, and expression of NF-κB target genes, including *IL-6*, *IL-10*, and *IL-15*, *GM-CSF*, and *interferon-gamma (IFN-γ)* (Grossman and Ratner, 1997; Portis *et al.*, 2001b). Primary tumor cells from these mice expressed IL-1, IL-6, IL-10, and IL-15, GM-CSF, and IFN-γ, but not IL-2, IL-4, or IL-9 (Grossman and Ratner, 1997). In contrast to primary tumor cells, tumor cell lines did not exhibit IL-1 expression, suggesting that IL-1 was expressed from a nonmalignant cell population infiltrating the LGL tumors. IL-1 can promote malignant cell growth and invasiveness, and also induce antitumor immunity (Apte and Voronov, 2002). High levels of GM-CSF in these transgenic mice may initiate neutrophilia, a characteristic finding for this animal model. IFN-γ expression is likely a manifestation of the activated NK cell phenotype and may contribute to angiostatic regulation of these tumors. GzmB-Tax transgenic mice with homozygous deletion of IFN-γ manifested earlier onset of tumors and enhanced angiogenesis, but no significant alteration in CD4+ or CD8+ immune responses (Mitra-Kaushik *et al.*, 2004a).

The roles of other cytokines in this model remain to be determined. IL-6 is a pleiotropic cytokine, acting as an acute-phase reactant that regulates differentiation, proliferation, and survival of a wide variety of cell types (Horn *et al.*, 2000). IL-10 suppresses inflammatory responses, and regulates the growth of NK and T cells, and other cell types (Scholz *et al.*, 1996; Tsuruma *et al.*, 1996; Furuya *et al.*, 1999; Carvalho *et al.*, 2001). Thus, IL-10 could play an immunoevasion role for HTLV-1 infection. The role of overexpressed ILs in HTLV-1 carcinogenesis remains unclear, but the future use of siRNAs and breeding with knockout mice should clarify their individual contributions. Inducible systems to test the requirement of Tax in tumor maintenance and allow differential expression of Tax *in vivo* are needed to verify the role of NF-κB in HTLV-1-mediated lymphoma.

The p53 tumor suppressor and cell cycle regulatory protein is mutated in some HTLV-1-associated lymphomas, and is functionally inactivated by Tax when exogenously expressed *in vitro* (Mahieux *et al.*, 2000; Pise-Masison *et al.*, 2001; Gatza *et al.*, 2003; Jeong *et al.*, 2004). In p53 heterozygous mice, tumor onset was the same as p53 homozygous wild-type animals, but the heterozygous animals manifested more rapid tumor progression and death (Portis *et al.*, 2001a). The molecular basis for this phenotype remains to be determined.

Therapeutic modalities in HTLV-1 animal models

Animal models of ATL have been quite useful in testing various anticancer therapeutic approaches. Based on the findings of NF-κB activation in ATL, several investigators have utilized these models to block NF-κB expression. The proteasome inhibitor, borteozomib, inhibits the degradation of the inhibitor of NF-κB, IκBα. It was shown to inhibit NF-κB activation in ATL cells and Tax transgenic tumor cells in culture and in mouse transplantation studies (Tan and Waldmann, 2002; Satou *et al*., 2004; Mitra-Kaushik *et al*., 2004b). In several of these studies, the majority of the cell death was found to be due to apoptosis. An inhibitor of NF-κB DNA-binding activity, Bay 11-7082, was also shown to block NF-κB activity and resulted in tumor regression in ATL-transplanted NOD-SCID-gammac knockout mice (Dewan *et al*., 2003).

Treatment of ATL-bearing mice with a humanized anti-CD2 monoclonal antibody led to tumor regression (Zhang *et al*., 2003b). The activity of the mononclonal antibody was most likely due to antibody-dependent cellular cytotoxicity, since expression of Fcγ receptors on neutrophils and monocytes was required for activity. Treatment of ATL-bearing NOD-SCID mice with an α-emitting radionuclide, bismuth 213, conjugated to an antibody to the IL-2 receptor proved to be highly effective in inducing tumor regressions (Zhang *et al*., 2002). The activity of bismuth 213 in this model was greater than that of unconjugated antibody or radionuclide, or antibody conjugated to β-emitting radionuclide, yttrium 90. Flavopiridol, an inhibitor of cyclin-dependent kinases, was tested for its therapeutic efficacy alone and in combination with humanized anti- Tac antibody (HAT), which recognizes CD25, in a murine model of human ATL using MET-1 leukemic cells (Zhang *et al*., 2005). Either flavopiridol, given 2.5 mg/kg body weight daily for 5 days, or HAT, given 100 μg weekly for 4 weeks, inhibited tumor growth and prolonged survival of the leukemia-bearing mice (Zhang *et al*., 2005). Collectively, these studies provide hope that ATL transplant and Tax transgenic models will provide new directions in the development of effective therapies against HTLV-1-induced lymphoproliferative diseases including ATL.

Future prospects

During the past 25 years since the first isolation of HTLV-1 from patients' cutaneous forms of ATL, a variety of animal models of HTLV-1 infection and transformation have expanded the understanding of the role of viral gene products and host factors that determine HTLV-1-mediated lymphocyte transformation. The knowledge gained from the study of animal models of HTLV-1 infection and disease can be extended to understand the pathogenesis and therapeutic intervention strategies of other tumors of hematopoietic origin. Future application of these animal models will also be critical in the development of vaccine approaches to elicit cellular immune responses to key viral proteins that mediated transformation, for example, Tax and to refine pharmacological targets to ablate HTLV-1-transformed T cells. Newer approaches using inducible expression systems (e.g. Cre lox recombinase) will need to be developed to address issues related to the role of viral gene products in the maintenance of the tumor phenotype in ATL. Moreover, recent studies of the HTLV-1 receptor suggest new approaches in using genetically engineered mouse models for HTLV-1 infection.

Acknowledgements

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Table 1

Animal models of HTLV-1 infection and disease

Animal[a]	Advantages	Disadvantages
Rabbit	Low cost and ease of maintenance Easy to induce persistent infection and test immune response, transmission routes, and parameters of infection, for example, viral loads Evaluation of viral genetic determinants of infection using HTLV-1 molecular clones	No reliable disease induced following infection Lack of broadly available reagents to study
Rat	Low cost, ease of maintenance, and can be genetically modified Can become persistent infected to test immune response (e.g. cytotoxic T cells) and parameters of infection, for example, viral loads Some strains useful for neurologic disease model Immunologic reagents available	Wide variation in infection in some strains Disease induced following infection does not histologically model human neurologic disease ATL models require immunodeficient rats
Immunocompetent mice	Low cost, ease of maintenance, and can be genetically modified Can test immune response (e.g. cytotoxic T cells) Immunologic reagents available	Limited evidence of *in vivo* spread of infection No apparent disease induced following infection
Non-human primate	Phylogenetic similarity to humans Good model for persistent infection Development of HTLV-like disease using molecular clones reported Immunologic reagents available	Cost and availability Inconsistent disease development after prolonged period of time
Immunodeficient mice (e.g. NOD/SCID)	Development of lymphoma following transplantation Used to test therapeutic reagents against tumor Immunologic reagents available	Cost and complicated maintenance Available of ATL cell lines to induce tumors is limited
Transgenic or knockout mice	Induction of lymphoma and other lymphocyte-mediated diseases using individual gene products of HTLV-1 Immunologic reagents available Ability to test of host parameters in induction of disease, for example, cytokines	Cost of production and maintenance CD4+ T-cell lymphomas inconsistently produced Overexpression of viral gene products may not be representative of carcinogenesis in HTLV-1 infection

[a]Excluding BLV and STLV animal models