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Inflammatory Polyarthropathy and Bone Remodeling in HTLV-I Tax-Transgenic Mice

Saggioro, Daniela*†; Rosato, Antonio*; Esposito, Gianni*; Rosenberg, Michael P.‡; Harrison, Jeanette§; Felber, Barbara K.§; Pavlakis, George N.§; Chieco-Bianchi, Luigi*

Author Information

** Institute of Oncology, Interuniversity Center for Cancer Research,† IST Biotechnology Section, University of Padova, Italy; ‡ Department of Pharmacology, Glaxo Wellcome Research and Development Institute, Research Triangle Park, North Carolina; § ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland, U.S.A.*

Address correspondence and reprint requests to Daniela Saggioro, Istituto di Oncologia, Università di Padova, via Gattamelata 64, 35128 Padova, Italy.

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Abstract

Transgenic mice carrying the tax gene of human T-cell lymphotropic virus type I displayed a high prevalence of arthropathy. The percentage of affected animals increased with age, reaching a peak of 43% at 20 months. Southern analysis of deoxyribonucleic acid (DNA) from tissue samples indicated that disease development was related to tax copy number. Histopathologic evaluation of the ankle joints disclosed deep erosion of the synovial lining, fibrous tissue proliferation together with angiogenesis, mononuclear cell infiltration, and activation of osteoclasts. Radiologic examination confirmed joint involvement and revealed bone architecture modifications. The phenotype exhibited by the affected animals closely resembles that of seronegative arthritis in humans, and may indicate tax protein as a causal agent of arthropathy observed in HTLV-I infected individuals.

Human T-cell lymphotropic virus type I (HTLV-I) is etiologically associated with adult T-cell leukemia(ATL)[1,2] and a degenerative neurologic disorder termed tropical spastic paraparesis, or HTLV-I-associated myelopathy (TSP/HAM)[3]. In addition, other diseases such as polymyositis, infectious dermatitis, uveitis and chronic arthritis have been described in HTLV-I-infected subjects[4-8]. The mechanism by which HTLV-I infection leads to ATL and other diseases remains to be understood.

The virus encodes a protein termed tax that regulates the transcription of viral genes through the HTLV-I long terminal repeat (LTR) promoter[9,10]. Tax also induces the expression of several cellular genes involved in growth and differentiation[11], as well as certain cytokines with proinflammatory or chemoattractant activity[12,13]. Due to its transactivating activity, Tax is considered to be the putative transforming product of HTLV-I. In addition, on the basis of its properties as a transcriptional modulator of cellular genes, Tax is thought to play a major role in other HTLV-I related diseases.

To correlate Tax protein function with HTLV-I pathogenesis *in vivo,* several lines of transgenic mice capable of expressing Tax have been generated using different subgenomic fragments containing the tax gene. Some of these mice develop mesenchymal tumors[14,15], large granular lymphocytic leukemia[16], Sjogren's-like syndrome[17], rheumatoid-like arthritis[18,19], and skeletal alterations[20].

This report describes a study of tax-transgenic mice that express the viral Tax protein under the control of the mouse metallothionein (MT) promoter. Clinical observation of tax-positive mice revealed a high incidence of polyarthropathy, which was not observed in tax-negative littermates. Histologic and X-ray analysis of the mice revealed that the disease involved the ankle, knee, tarsal, metatarsal, and toe joints, as well as the caudal vertebrae. The disease was progressive and was related to the Tax copy number, thus suggesting a role for tax protein in human HTLV-I-associated chronic arthritis.

MATERIALS AND METHODS

Animals

The transgenic mice used in this study were generated by injecting C57BL/6 embryos with a linear deoxyribonucleic acid (DNA) fragment obtained by digesting the plasmid Max[21] with restriction enzymes Eco RI and Bam HI. The resulting 5.9-kb fragment expresses Tax as a hybrid protein consisting of seven amino acids of the amino terminus of murine MT linked to the tax ORF, under the control of the MT promotor (Fig. 1). This strategy was chosen in order to allow induction of high-level expression of the Tax protein upon stimulation of the MT promoter in the presence of heavy metals such as $ZnCl_2$ or $CdCl_2$. Although we did not administer heavy metals to the mice, they were found to express significant levels of Tax mRNA (see Results). A transgenic line, designated TG1335, was generated from an MT-tax founder mouse. Progeny of brother X sister crosses of the TG1335 mice exhibited high neonatal mortality and poor growth. Therefore, to maintain and expand the colony, we crossed 3 pairs of TG1335 mice with nontransgenic C57BL/6 mice. The resulting hemizygous F1 mice were mated to generate F2 (homozygous, hemizygous, or wild type tax-negative) mice or were backcrossed with the parental transgenic mice to generate Bc1 (homozygous and hemizygous) mice. The transmission of the Tax transgene in progeny mice was monitored by performing Southern analysis of DNA extracted from tail tissues (see below). Although high neonatal mortality was still observed for the homozygous progeny mice, no substantial early mortality was noted in the other crosses. All of the animals were kept under observation.

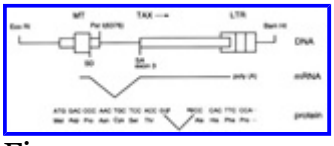

Fig. 1

DNA Extraction and Southern Blot Analysis

High molecular weight DNA was extracted from tail tissues using phenol: chloroform (1:1, v/v). DNA (10 μg) was digested with the restriction enzyme Sst I (Boehringer Mannheim, Germany), and then fractionated by electrophorsis on 0.8% agarose gels, denatured, neutralized, and transferred to Nytran membranes(Amersham, England), according to the method of Southern[22]. All filters were hybridized with a tax-specific probe to verify the presence of the transgene. The number of copies of the tax gene transmitted to each mouse was determined by hybridizing blots simultaneously with DNA probes specific for tax and the cellular mos gene (c-mos), used in approximately equivalent activities; the relative intensities of the tax and c-mos bands were then compared by analysis of the autoradiograms by scanning densitometry. The tax probe was obtained by PCR amplification as described[23]; the mos probe was derived from the v-mos-encoding plasmid MSV-31 using restriction endonuclease Pst I[24], and was highly efficient in detecting the c-mos gene.

Clinical Evaluation

Animals were kept under observation from birth to 24 months of age, and monitored weekly for assessment of clinical signs and symptoms as described in the text.

Histologic and Immunologic Analysis

Animals were sacrificed by administration of ether and then bled. The principal organs were removed and processed for isolation of ribonucleic acid (RNA) (see below) or fixed in 10% buffered formalin and embedded in paraffin. The limbs (toes, knees, and ankles) and the tails were immediately frozen in liquid nitrogen and stored at -80°C until they were processed. Cryostatic limb and tail specimens were decalcified using Kristensen solution (8 N formic acid and 1 N sodium formate, 1:1), fixed in 10% buffered formalin, and embedded in paraffin. Formalin-fixed samples were stained with hematoxylin and eosin or PAS (Periodic Acid-Schiff reactive).

Levels of inflammatory cytokines and Ca^{++} were analyzed in mouse serum. TNF-α and IL-6 levels were measured in biologic assays utilizing WEHI 164 and 7TD1 cell lines, respectively, as indicators[25,26]. IL-1β concentration was measured by ELISA(Genzyme Diagnostics, Cambridge, MA). Ca^{++} was tested using a colorimetric assay (AB CC-02 kit, AB Analytical). The sera were stored in small aliquots at -20°C in order to avoid sample degradation.

RNA Extraction and RT-PCR Analysis

Total RNA from mouse organs and tissues was isolated by the guanidine isothiocyanate method[22]; prior to processing, care was taken to eliminate extraneous tissue from the structure of interest. Total RNA (1.5 μg) was reverse transcribed using avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim, Germany) in the presence of 500 ng of random hexamers (Pharmacia, Uppsala, Sweden) for 2 hours at 45° C. Ten μl of reverse transcription reaction were amplified using the following primers: 5'-ATG GAC CCC AAC TGC TCC AC-3' (forward), specific for the 5' end of the mouse MT ORF (Fig. 1), and 5'-GGC GGG GTA AGG ACC TTG A-3' (reverse) corresponding to nucleotides 7205-7223 of the tax gene. The expected size of the PCR product was 293 bp.

HTLV-I numbering is according to Seiki et al.[27], in which +1 is the first nucleotide of the viral mRNA at the beginning of the R region.

RESULTS

Tax Detection

The tax-transgenic mice used in this study were derived from the C57BL/6 strain and carry a DNA fragment that encodes Tax with seven amino acids of mouse MT at its amino terminus, driven by the MT promoter (Fig. 1; see Materials and Methods). Sibling crosses of the homozygous line, designated TG1335, resulted in a high rate of neonatal mortality and poor growth, with only a few mice reaching sexual maturity. To maintain and expand the colony, we crossed the TG1335 mice with normal C57BL/6 (B6) mice to generate F1 offspring; we then performed backcrosses (Bc) with parental homozygous mice or sibling matings. Assuming a Mendelian transmission of the transgene, we expected these breedings to produce tax-homozygous mice, tax-hemizygous mice and tax-negative mice. To verify the presence of the tax gene in the progeny, genomic DNA extracted from the tails of individual animals was analyzed by the Southern blot technique. The relative tax copy number was evaluated by hybridizing some blots simultaneously with tax and c-mos probes. As the c-mos gene is present as single copy per haploid genome, the mos probe would serve as a standard to quantitate the relative copy number of the tax transgene; thus, the ratio between the optical density of tax and c-mos specific bands, determined by densitometric analysis, enabled us to estimate the relative tax copy number present in the genome of transgenic mice. We found that the mice possessed the expected copies of the tax gene per genome, depending on the cross we performed. Five representative crosses are reported in Figure 2. The tax-negative littermates were kept under observation along with the tax-positive mice and used as controls.

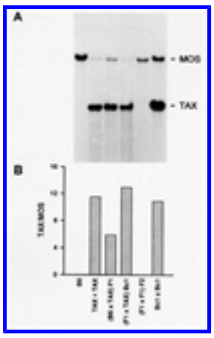

Fig. 2

RT-PCR analysis showed that the tax mRNA was expressed in several tissues including striated muscle, kidney, testis, brain, spleen, lymph nodes, lung, and liver of young adults of both sexes (Fig. 3); tax mRNA was also detected in thymus and salivary glands. Additional RT-PCR assays of joint specimens (tail, knee, ankle) confirmed expression of the tax mRNA in these structures. RT-PCR assays to detect other HTLV-I mRNA that could be derived from the MT-Tax gene by alternative splicing were negative, indicating that the expected MT-Tax mRNA is the only one produced in the mice (data not shown).

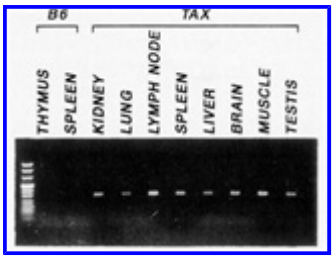

Fig. 3

To test the expression and function of the transgenic Tax protein, we derived fibroblast-like cell lines from tail tissue of homozygous and hemizygous mice. Results of Western blots confirmed expression of Tax, and results of transfection assays using an HTLV-I LTR CAT reporter plasmid confirmed that the protein was functionally active (Saggioro et al., manuscript in preparation). These results verify previous studies of biologic activity of Tax produced from the same plasmid used to generate the transgenic mice[9,21].

Clinical Observation

Clinical evaluation was carried out weekly for 24 months. Among 162 tax-positive and 37 tax-negative littermates studied, a high number of tax-positive mice presented limb abnormalities (i.e., erythema and swelling of the footpads and ankles) (Fig. 4). Although these abnormalities were evident in a few mice at the age of 3-5 months, the number of affected animals increased in mice up to 20 months of age, when a peak cumulative incidence of 43% was reached (Fig. 5). Animals were considered positive for the disease if erythema and swelling were present in at least one footpad; the graph shown in Figure 5 presents those animals in which the disease had progressed to a more advanced stage and involved several joints. Many small and large joints were involved, indicating that the inflammation was polyarticular. The disease progression was slow, and initially involved the hindlimbs and the tail, and only in a more advanced stage, the forelimbs. In some cases, the erythema and swelling were accompanied by rigidity of the knee and ankle joints. The incidence of arthropathy was not sex-linked but appeared to be related to the tax copy number present in the genome(i.e., homozygousity versus hemizygousity). Indeed, analysis of the arthropathy prevalence in some mice disclosed that almost 74% of the tax-homozygous mice developed polyarthropathy by 24 months of age, while disease prevalence in hemizygous mice was much lower (Table 1). However, a high tax copy number did not appear to influence the age of onset or to accelerate the progression of the disease. None of the mice developed tumors.

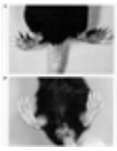
Fig. 4

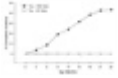
Fig. 5

Table 1

X-ray Examination

Radiologic analysis confirmed the involvement of several joints and also revealed the presence of morphologic and structural alterations in bone architecture. In comparison to age-matched tax-negative littermates (Fig. 6A), the arthropathy-affected tax-positive mice exhibited degeneration of metatarsophalangeal and interphalangeal joints of the hindlimbs, including severe narrowing of the interarticular space. Bones appeared swollen and acquired a spongeal aspect, with loss of differentiation between the cortical and spongeal areas due to resorption of the latter into the subcortical area (Fig. 6B, 6C).

Erosive changes in the phalanx bones were also visible, and in some cases calcification of ligaments (Fig. 6B) and spur formation (Fig. 6C) were also observed. Disruption and collapse of the osseous and/or articular structures were evident in the caudal vertebrae (Fig. 6D). With disease progression, the forelimbs also became involved and showed articular and osseous changes similar to those seen in the hindlimbs(data not shown).

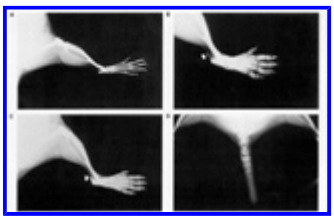

Fig. 6

Serologic analysis did not reveal a correlation between polyarthropathy development and the presence of rheumatoid factor; indeed, among five mice showing severe polyarthropathy, only two were positive for rheumatoid factor at a low titre. In addition, serum samples from the mice were analyzed to determine the levels of the inflammatory cytokines IL-6, TNF-α, and IL-1β. All of the samples tested negative for IL-6 (less than 50 U/ml). The serum levels of TNF-α and IL-1β varied among the mice, with TNF-α values ranging from 10 to 120 U/ml and IL-1β levels ranging from 1.2 to 32.5 pg/ml. However, TNF-α and IL-1β levels did not show a pattern connected to clinical status or to the presence of tax transgene. Thus, in our experimental model, no correlation was found between disease development and serum levels of the tested cytokines.

Hypercalcemia is often associated with ATL[28,29]. Interestingly, Tax has been shown to activate expression of parathyroid hormone-related peptide(PTHrP)[30], which plays a primary role in bone resorption and the development of humoral hypercalcemia. Thus, we also analyzed the Ca^{++} level in the serum of tax-transgenic mice; however, no correlation between disease and serum Ca^{++} values was observed.

Histologic Examination

Histologic analysis of advanced articular lesions disclosed erosion of the synovial lining, destruction of the cartilage and pannus formation, and the growth of fibrous tissue with newly formed capillaries mixed with mononuclear inflammatory cell infiltrates (Fig. 7A, 7B). In some specimens, synovial lining proliferation and thickening were detected. The lesions were most evident in the ankles and toe joints. The toe joints showed bone resorption with prominent fibrous tissue proliferation and osteoclast activation(Fig. 7C, 7D). The presence of osteoblasts was also observed along the bone speculae (Fig. 7C), indicating a significant level of bone remodeling. Bone architecture destruction, mononuclear cell infiltration, and growth of fibrous tissue were also found in sections taken from the proximal part of the tail (data not shown). Immunohistochemical analysis of frozen specimens using specific monoclonal antibodies disclosed that the infiltrating lymphocytes were mostly $CD4^+$ cells, with a minor component of $CD8^+$ cells. No inflammatory cells were detected in other tissues such as muscles and salivary glands, which have been reported to be involved in a Sjogren's-like syndrome observed in other tax-transgenic mice[17]. The kidneys of older animals often showed perivascular lymphocyte infiltration, hyalinosis of the glomeruli, and foci of ectasic tubuli with a thyroid-like appearance. However, as this chronic pyelonephritis-like pathology was also observed in some aged tax-negative littermates, we could not conclusively link it to tax gene expression.

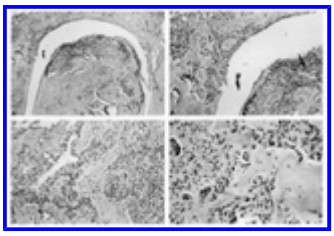

Fig. 7

DISCUSSION

In addition to inducing ATL and neurologic disorders(TSP/HAM), HTLV-I infection has been correlated with a variety of diseases such as polymyositis, uveitis, arthritis, and other autoimmune disorders. Due to its transactivating activity, it is commonly held that the viral Tax protein not only is the transforming product of HTLV-I, but might also be involved in these additional HTLV-I-related diseases. The potential pathogenic character of Tax is supported by our observation that transgenic mice expressing the Tax protein develop inflammatory polyarthropathy at a high incidence, which is accompanied by destruction of bone structure.

The presence of inflammatory arthropathy has previously been described in HTLV-I-transgenic mice by Yamamoto and co-workers[19]. It is interesting that although their mice carry a larger portion of the HTLV-I genome (LTR-env-pX region) compared to our mice, the two lines develop similar diseases. However, in describing arthritis development in their transgenic mice, Yamamoto et al. found a major involvement of large joints. In contrast, our tax-transgenic mice initially show a significant involvement of small joints of the hindlimbs and vertebrae; the large joints become involved only later, as the disease progresses. In addition, the LTR-env-pX transgenic mice were not reported to display bone architecture modifications, which are an important feature of our tax-transgenic line. In fact, bone destruction was evident both by x-ray analysis of pathologic limbs and upon histologic examination of toe specimens, which revealed the presence of numerous activated osteoclasts and osteoblasts, indicating bone remodeling. It is noteworthy that bone turnover associated with osteoclast and osteoblast activation has been described in another line of tax-transgenic mice[(20)].

In our tax-transgenic mouse model, it is difficult to assess whether the first lesions arise at the joint or at bone tissue level, and no correlation between the appearance of inflammatory arthropathy and the presence of rheumatoid factor could be found. The development of the disease is clearly linked to the presence of the tax gene in the genome, as no arthropathy or bone destruction was detectable among 37 tax-negative littermates by clinical, x-ray, and histologic examination. Furthermore, we found that tax-homozygous mice developed the disease with a statistically significant higher incidence than hemizygous mice. Taken together, these observations indicate a real involvement of Tax protein in the development of the observed phenotype. The fact that a comparable phenotype was described in animals generated with a different construct capable of expressing Tax[(18,19)] argues against the possibility that the phenotype is the mere result of insertional mutagenesis.

Tax induces expression of a variety of cellular genes, including those encoding proinflammatory cytokines such as TNF, granulocyte/macrophage-colony stimulating factor (GM-CSF), IL-1, and IL-6[(12,31-33)]. Thus it is possible that the chronic inflammation found in our transgenic mice might develop as a result of overexpression of one or more cytokine(s) induced by Tax. In fact, elevated expression of various inflammatory

cytokines and autoantibodies has been reported in HTLV-I transgenic mice[34]. However, to date, no correlation between the presence of inflammatory cytokines in the serum of our animals and the disease has been found; likewise, there appears to be no link between the production of autoantibodies and their arthropathy (Dr. S. Muller, CNRS, Strasbourg, France, personal communication).

Two groups have recently hypothesized the involvement of overexpression of proto-oncogenes together with the activation of cellular genes implicated in cell growth or apoptosis, in a patogenetic network leading to tissue destruction in human arthritis[35,36].

Our data, together with the documented presence of chronic inflammatory arthropathy in patients infected with HTLV-I[8], implicate HTLV-I, through its Tax product, as one of the putative etiologic coagents of human arthritis in infected individuals. Due to its transactivating capacity, one can envisage that Tax plays an important role in the proposed protooncogene network. Furthermore, our findings on bone architecture destruction and remodeling might indicate a role of Tax in the skeletal abnormalities observed in ATL patients[26,27]. Further analyses of the tax-transgenic mice will lead to the identification of the molecular targets of Tax protein that are involved in these pathologies.

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REFERENCES

1. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980;77:7415-9.

2. Hinuma Y, Nagata K, Hanaoka M, et al. Adult-T cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA* 1981;78:6476-80.

3. Gessain A, Vernant JC, Maurus L, et al. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 1985;2:407-10.

4. Morgan OS, Rodgers-Johnson P, Mora C, Char G. HTLV-I and polymyositis in Jamaica. *Lancet* 1989;2:1184-6.

5. Leon-Monzon M, Illa I, Dalakas MC. Polymyositis in patients infected with human T-cell leukemia virus type I: the role of the virus in the cause of the disease. *Ann Neurol* 1994;36:643-9.

6. Yamaguki K, Mochizuki M, Watanabe T, et al. Human T-lymphotropic virus type I uveitis. *Leukemia* 1994;8:s88-s90.

7. Sagamura K, Mochizuchi M, Masuoka K, et al. Immunopathological mechanisms of human T cell lymphotropic virus type I (HTLV-I) uveitis. Detection of HTLV-I-infected T cells in the eye and their constitutive cytokine production. *J. Clin Invest* 1995;95:852-8.

8. Nishioka K, Nakajima T, Hasunuma T, Sato K. Rheumatic manifestation of human leukemia virus infection. *Rheum Dis Clin North Am* 1993;19:489-503.

9. Felber B, Paskalis H, Kleinman-Ewing C, Wong-Staal F. Pavlakis GN. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. *Science* 1985;244:675 -9.

10. Seiki M, Inoue J, Takeda T, Yoshida M. Direct evidence that $p40^{x}$ of human T-cell leukemia virus type I is a *trans*-activating transcriptional activator. *EMBO J* 1986;5:561-5.

11. De Rossi A, Ciminale V, Saggioro D, D'Agostino D, Chieco-Bianchi L. Oncogenesis by the human T lymphotropic virus type I: molecular and biological features. *Forum* 1994;4:266-84.

12. Greenberg SJ, Tendler CL, Manns A, et al. Altered cellular gene expression in human retroviral-associated leukemogenesis. In: Blattner WA, ed. *Human Retrovirology: HTLV*. New York: Raven Press, Ltd, 1990:87-104.

13. Saggioro D, Wang JM, Sironi M, Luini W, Mantovani A, Chieco-Bianchi L. Chemoattractant(s) in culture supernatants of HTLV-I-infected T cell lines. *AIDS Res Hum Retroviruses* 1991;7:571-7.

14. Nerenberg M, Hinrichs SH, Reynolds RK, Rhoury G, Jay G. The tax gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. *Science* 1987;237:1324-9.

15. Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G, Jay G. A transgenic mouse model for human neurofibromatosis. *Science* 1987;237:1340-3.

16. Grossman WJ, Kimata JT, Wong F-H, Zutter M, Ley TJ, Ratner L. Development of leukemia in mice transgenic for the *tax* gene of human T-cell leukemis virus type I. *Proc Natl Acad Sci USA* 1995;92:1057-61.

17. Green JE, Hinrichs SH, Vogel J, Jay G. Exocrinopathy resembling Sjogren's syndrome in HTLV-1 tax transgenic mice. *Nature* 1989;341:72-4.

18. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 1991;253:1026-8.

19. Yamamoto H, Sekiguchi T, Itagaki K, Sajio S, Iwakura Y. Inflammatory polyarthritis in mice transgenic for human T cell leukemia virus type I. *Arthritis Rheum* 1993;36:1612-20.

20. Ruddle NH, Li C-B, Horne WC, et al. Mice transgenic for HTLV-I LTR-tax exhibit tax expression in bone, skeletal alterations and high bone turnover. *Virology* 1993;197:196-204.

21. Paskalis H, Felber BK, Pavlakis GN. Cis-acting sequences responsible for the transcriptional activation of human T-cell leukemia virus type I constitute a conditional enhancer. *Proc Natl Acad Sci USA* 1986;83:6558-62.

22. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning*, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.

23. Calabro' ML, Luparello M, Grottola A, et al. Detection of human T lymphotropic virus type II/b in human immunodeficiency virus type I-coinfected persons in southeastern Italy. *J Infect Dis* 1993;168:1273-7.

24. D'Andrea E, Tessarollo L, Menin C, Chieco-Bianchi L. Tumours induced by Moloney murine sarcoma virus are clonal in rats, not clonal in mice. *J Cancer Res Clin Oncol* 1992;118:141-6.

25. Monastra G, Cabrelle A, Zambon A, et al. Membrane form of TNFα induces both cell lysis and apoptosis in susceptible target cells. *Cell Immunology* 1996;171:102-10.

26. Sironi M, Breviario F, Proserpio P, et al. Il-1 stimulates Il-6 production in endothelial cells. *J Immunol* 1989;142:549-53.

27. Seiki M, Hattori S, Hirayama Y, Yoshida M. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc Natl Acad Sci USA* 1983;80:3618-22.

28. Kiyokawa T, Yamaguchi K, Takeya M, et al. Hypercalcemia and osteoclast proliferation in adult T-cell leukemia. *Cancer* 1987;59:1187-91.

29. Prager D, Rosenblatt JD, Ejima E. Hypercalcemia, parathyroid hormone-related protein expression and human T-cell leukemia virus infection. *Leuk Lymphoma* 1994;14:395-400.

30. Watanabe T, Yamaguchi K, Takatsuki K, Osame M, Yoshida M. Constitutive expression of parathyroid hormone-related protein gene in human T cell leukemia virus type I (HTLV-I) carriers and adult T cell leukemia patients that can be transactivated by HTLV-I tax gene. *J Exp Med* 1990;172:759-65.

31. Nakajima T, Kitamura K, Yamashita N, et al. Constitutive expression and production of tumor necrosis factor-beta in T-cell lines infected with HTLV-I and HTLV-II. *Biochem Biophys Res Commun* 1993;191:371-7.

32. Dao T, Holan V, Minowada J. Multiple and heterogeneous patterns of cytokine production in 18 leukemia and in vivo transformed mature T cell lines reflect the individuality of human leukemias. *Int J Hematol* 1993;57:139-46.

33. Lal RB, Rudolph D, Buckner C, Pardi D, Hooper WC. Infection with human T-lymphotropic viruses leads to constitutive expression of leukemia inhibitory factor and interleukin-6. *Blood* 1993;81:1827-32.

34. Iwakura Y, Sajio S, Kiota Y, et al. Autoimmunity induction by human T cell leukemia virus type I in transgenic mice that develop chronic inflammatory arthropathy resembling rheumatoid arthritis in humans. *J Immunol* 1995;155:1588-98.

35. Carson AD, Ribeiro MJ. Apoptosis and disease. *Lancet* 1993;341:1251-54.

36. Muller-Ladner U, Kriegsmann J, Gay ER, Gay S. Oncogenes in rheumatoid arthritis. *Rheum Dis Clin North Am* 1995;21:675-90.

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