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The Human T Cell Leukemia Virus Type I-tax Gene Is Responsible for the Development of Both Inflammatory Polyarthritis Resembling Rheumatoid Arthritis and Noninflammatory Anklyotic Arthritis in Transgenic Mice

Kiyoshi Habu,† Junko Nakayama-Yamada,* Masahide Asano,* Shinobu Saijo,* Keiko Itagaki,* Reiko Horai,* Hiroaki Yamamoto,† Toyozo Sekiguchi,† Tetsuya Nosaka,‡ Masakazu Hatanaka,‡ and Yoichiro Iwakura2*  

We previously reported that inflammatory arthritis resembling rheumatoid arthritis (RA) develops among transgenic mice carrying the long terminal repeat (LTR)-env-pX-LTR region of human T cell leukemia virus type I (LTR-pX-Tg mice). Because four genes are encoded in this region, we produced transgenic mice that only express the tax gene to examine its role in the development of arthritis. Transgenic mice were produced by constructing DNAs that express the tax gene alone under the control of either its own LTR or CD4 enhancer/promoter and by microinjecting them into C3H/HeN-fertilized ova. We produced seven transgenic mice carrying the LTR-tax gene and nine mice carrying the CD4-tax and found that one of the LTR-tax-Tg mice and five of CD4-tax-Tg mice developed RA-like inflammatory arthropathy similar to LTR-pX-Tg mice, indicating that the tax gene is arthritogenic. On the other hand, the other two LTR-tax-Tg mice had anklyotic changes caused by new bone formation without inflammation. In these anklyotic mice, tax mRNA, inflammatory cytokine mRNA, and autoantibody levels except for TGF-β1 level were lower than those in LTR-pX- or CD4-tax-Tg mice. These results show that Tax is responsible for the development of inflammatory arthropathy resembling RA and that this protein also causes anklyotic arthropathy. *The Journal of Immunology, 1999, 162: 2956–2963.

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uman T cell leukemia virus type I (HTLV-I)§ is the etiologic agent of adult T cell leukemia (1). This virus encodes a transcriptional transactivator, Tax, in the pX region (for review, see Ref. 2). The Tax protein is known to transactivate transcription from the cognate viral promoter as well as the CD4 and IL-2 promoters, indicating that Tax is an oncogene (2). The Tax protein has been postulated that the transactivation activity of Tax may be responsible for the development of diseases caused by this virus (2).

In a previous paper, we produced the long terminal repeat (LTR)-pX-Tg mice that carry the HTLV-I env-pX region under its own LTR promoter and showed that these mice develop chronic inflammatory polyarthropathy (13). The arthritis developed as early as 4 wk of age and increased in incidence gradually after that. By 3 mo of age about 20% of the mice and by 6 mo 40% of the mice (C3H/HeN background) were affected. The affected mice were easily detected by swelling and redness of the joints with lesions most frequently seen in the hind leg ankles. The histopathology of the lesions showed marked synovial and periarticular inflammation with articular erosion caused by the invasion of granulation tissues (14).

Immunologically, these mice produced autoantibodies against IgG, type II collagen (IIC), and heat shock proteins, and had accompanying IgG hypergammaglobulinemia (15). Cellular immune response to IIC as well as to heat shock proteins was also activated. In addition, the N-linked sugar chains of the transgenic IgG had more galactosylated forms than those from nontransgenic IgG, as is seen in patients with rheumatoid arthritis (RA) (16). We also found that genes for inflammatory cytokines including IL-1α, IL-1β, IL-6, TNF-α, TGF-β, IFN-γ, and IL-2 as well as MHC genes were activated in the transgenic joints (15). Because these pathological observations were very similar to those of RA, we suggested that HTLV-I may be involved in the development of RA in humans (13). However, it remains to be elucidated which gene in the pX region is involved in the development of the disease, as the transgene of LTR-pX-Tg mice consisted of the env, tax, rex, and p21 genes.

In the current study, to elucidate the pathogenesis of the disease, we tested the involvement of the tax gene in disease pathogenesis by producing transgenic mice that expressed only the tax gene under two different enhancer/promoters. The results showed that tax expression by itself induced arthropathy resembling RA. Interestingly, two founder mice developed anklyotic arthropathy with bone hyperplasia.

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§Abbreviations used in this paper: HTLV-I, human T cell leukemia virus type I; LTR, long terminal repeat; LTR-pX-Tg mouse, transgenic mouse carrying the HTLV-I env-pX region under its own LTR promoter; LTR-tax-Tg mouse, transgenic mouse carrying the HTLV-I tax gene under its own LTR promoter; CD4-tax-Tg mouse, transgenic mouse carrying the HTLV-I tax gene under the murine CD4 promoter enhancer; RA, rheumatoid arthritis; IIC, type II collagen; m, murine.
but without inflammation, which is a considerably different pathology from that seen in RA.

Materials and Methods

Materials

Rabbit IgG was purified from serum by ammonium sulfate precipitation followed by protein A column chromatography and heat denatured at 62°C for 20 min. Bovine IIC (K42) was obtained from Collagen Gijyutsu-ken-shukai (Tokyo, Japan).

Plasmids

pLTR-tax was constructed by ligating a 4.0-kb HindIII fragment containing the HTLV-I 5′LTR region of pHLX-1 (13) to a 2.5-kb HindIII fragment of pKCR40M (17) containing the HTLV-I pX and 3′LTR regions (see Fig. 1A). The pX region of pKCR40M was constructed so as to produce only Tax protein as described previously (17). In brief, the r ex gene was disrupted by deleting its initiation codon, and the p21 gene was also disrupted by changing the initiation codon at nucleotide 7477 from ATG to ACG, whereas tax mRNA is intact because its initiation codon was supplied by a synthetic oligonucleotide (see Fig. 1B). To construct pCD4-tax, a 0.85-kb HindIII-XhoI fragment from the mouse CD4 enhancer/promoter (pBLD4C/0.35 wt) (18) and a 0.64-kb HindIII-BamHI fragment from the rabbit β-globin gene (1757) (19) were ligated, then a 1.59-kb HindIII-BamHI fragment from this construct, and a 2.3-kb BamHI-BglII fragment from pKCR40M, containing the tax gene, were inserted into HindIII-BglII site of p1017 (20), which provides an extra poly(A) additional signal of the human growth hormone gene (see Fig. 1C).

Production of transgenic mice

LTR-tax-Tg mice were produced by injecting a 3.2-kb EcoRI fragment from pLTR-tax into fertilized C3H/HeN mouse ova. The founder mouse was crossed with C3H/HeN, and the homzygotes were produced by intercrossing the F2 mice. The line was maintained by sister-brother mating. In the case of CD4-tax-Tg mice, a 4.6-kb SphI fragment was injected into fertilized C3H/HeN ova. The LTR-pX-Tg mice (line T647) were those previously described by our group (13). These mice were produced by introducing an EcoRI fragment from pHLX-I containing the env-pX region of the HTLV-I genome with its own promoter into fertilized mouse ova (C3H/HeN x C57BL/6J)F1, backcrossing each of the mice with a C3H/HeN mouse. Mice from backcross generation 10–12 were used for the experiments. Female mice at the age of 2–3 mo were used in this study, except for certain experiments described in the figure legends, and the littermates were used as controls. The transgenes were detected through dot-blot hybridization using DNA prepared from mouse tails and 32P-labeled transgene as the probes (15). These mice were kept in specific pathogen-free conditions in environmentally controlled clean rooms of the Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo (Tokyo, Japan). The experiments were conducted according to the institutional ethical guidelines for animal experiments and safety guidelines for gene manipulation experiments in Japan.

Southern blot hybridization

Genomic DNA (15 μg) prepared from the liver was digested with Mbol and Drai, and after electrophoresis on a 0.8% agarose gel, it was transferred to nylon membranes (GeneScreen Plus, DuPont/NEN). Hybridization was conducted by 32P-labeled 0.76-kb AccI-SalI fragment (probe A in Fig. 1A) from pLTR-taxe as the probe (1 x 106 dpm/μg) at 42°C for overnight, then membranes were washed twice with 2x SSC plus 1.0% SDS at room temperature and three times with 0.1x SSC plus 1.0% SDS at 65°C. The intensity of the bands on the autoradiogram was estimated by BAS 2000 system (Fuji Photo Film, Kanagawa, Japan).

Detection of mRNA

Cytokine mRNAs were analyzed by Northern blot hybridization as previously described (15). Briefly, total RNA was prepared from various tissues using acid guanidinium thiocyanate-phenol-chloroform method (21) and poly(A) + RNA was purified from the total RNA with an oligo(dT)-cellulose column (QuickPrep, Pharmacia Biotech, Piscataway, NJ). For thigbone RNA preparation, bone marrow cells and muscles and skin were removed from the bone. For Northern blot hybridization, 5 μg poly(A)-RNA was electrophoresed on a denatured agarose gel. Then, RNA was transferred onto a nylon membrane filter and hybridized at 42°C for overnight with a 32P-labeled DNA probe (108 dpm/μg) labeled with [α-32P]dCTP (3000 Ci/mmol; DuPont/NEN) by a multiprime DNA labeling system (Amersham, Arlington Heights, IL).

Tax mRNA was detected by RNase protection assay (13). Total RNA purified from pooled tissues from two mice (40 μg) or from MT-2 cells (13) (0.1 μg) was hybridized at 50°C overnight with an antisense RNA probe (837-base) made with T7 RNA polymerase using the AccI-Sal fragment (probe B in Fig. 1A) from pLTR-taxe that had been subcloned into the EcoRV site of pBluescript KS (+) (Stratagene, La Jolla, CA) as a template. After RNase A (40 μg/ml) and T1 (2 μg/ml) treatment at 37°C for 1 h, the RNA samples were electrophoresed on a 4% polyacrylamide gel containing 7.8 M urea. Tax mRNA was detected as a 758-base band in the RNase protection assay. The intensity of the bands on the autoradiogram was estimated by the BAS 2000 system (Fuji Photo Film).

Detection of mRNA

The probes used in this study were previously described (15). Briefly, the XhoI fragments from CDMmIL-1α (2.0 kb) (22) and CDMmIL-1β (1.3 kb) (23) were used to detect IL-1α and IL-1β. IL-6 was probed with the BamHI (1.2 kb) fragment from moldL-6 (24), and TNF-α was detected with the BglII fragment (711 bp) from moTNF-α (25). The EcoRI-XhoI rat TGF-β1 cDNA clone (pBluescript TGF-B1) was used for a probe (11). β-Actin was detected using the Psfl fragment (1.0 kb) from pAL41 (26) and used as a control.

Histological examination

Tissues were fixed with 10% formalin in PBS and decalcified by treatment with Plank-Rycole solution (50% formic acid, 3% HCl, 0.3 M NH4Cl). Four-micrometer sections were prepared after embedding in paraffin and were stained with hematoxylin and eosin.

Ab titration

Serum IgG and IgM levels were determined as previously described (27). Serum Ab levels were measured by ELISA (15, 28). Briefly, polyclonal microtiter plates (Falcon MicroTest III; Becton Dickinson, Tokyo, Japan) were coated with either heat-denatured rabbit IgG (10 μg/ml) or bovine IIC (20 μg/ml). The plates were blocked with 1% skim milk (Sigma, St. Louis, MO) and 5 mM EDTA, and diluted mouse serum was added to each well and incubated for 1 h at room temperature. After washing, alkaline phosphatase-conjugated goat anti-mouse IgG Ab (Zymed, San Francisco, CA) or anti-mouse IgM Ab (Zymed) was added and incubated at 37°C for 1 h, followed by staining with p-nitrophenylphosphate (Sigma). The absorbancy at 415 nm was measured after incubation at 37°C for 1 h.

Protein concentration was measured with a Bradford Protein Determination kit (Bio-Rad, Hercules, CA) and BSA was used as a standard.

Results

Production of transgenic mice

We previously reported on LTR-pX-Tg mice that develop inflammatory arthropathy resembling RA. Because four genes are expected to be expressed in these transgenic mice, we produced transgenic mice that only express the tax gene to examine its role in the development of arthritis. For this, we constructed two types of transgenes; in one construct, pLTR-taxe, the tax gene was placed under the control of its own LTR promoter, and in the other construct, pCD4-taxe, the tax gene was ligated downstream to the CD4 enhancer/promoter (Fig. 1).

Seven LTR-taxe-Tg mice were produced by injecting the LTR-taxe DNA into the pronuclei of 3891 fertilized mouse ova. Three of these mice developed abnormalities of the joints. One of them, T3376 (male), showed remarkable swelling of the digital and ankle joints both of the forelegs and hind legs. This mouse died without producing an offspring. Another, T5571 (male), developed deformities of the left hind leg digital joints and the right ankle joint at 9 mo of age. This mouse showed ankylotic change of the ankle and knee joints later. This mouse also died at 12 mo of age without reproducing. T4055 (male) showed ankylotic changes in the joints without swelling, which was in contrast to those symptoms seen in T3376. None of the other mice showed any obvious joint changes. Thus, T3376 and T5571 founder mice and descendants of T4055 were analyzed further.
In case of CD4-tax-Tg mice, nine transgenic founder mice were produced by injecting the CD4-tax DNA into 1483 fertilized ova. Among them, five mice showed joint abnormalities with swelling of the ankle joints and redness of the foot pads before 3 mo of age. We established three lines, T1887, T5368, and T5562, from those mice with joint abnormality (Fig. 2B). Transgenic mice from these lines looked unhealthy, had smaller body size compared with the normal littermates, and did not breed very well. Because all of these lines showed similar pathology, we analyzed T5368 line mice in detail.

To verify integration of the tax gene in the host chromosome, the tail DNA was digested with MboI and DraI, which cut out a 1.3-kb fragment from the transgenes and was analyzed by Southern blot hybridization. As shown in Fig. 3, a 1.3-kb band of expected length was detected in both T5368 line CD4-tax-, T4055 line LTR-tax-, and LTR-pX-Tg mouse indicating integration of the transgene into the host chromosome. The copy numbers were estimated to be ~7, 1, and 50 copies per genome in T5368 line CD4-tax-, T4055 line LTR-tax-, and LTR-pX-Tg mice, respectively.

**Inflammatory polyarthropathy in tax transgenic mice**

Joint swelling of T3376 LTR-tax-Tg mouse started at the age of 1 mo and continued throughout life. His growth was markedly retarded, and his body weight was 7.3 g at 2 mo old, about one-third the weight of normal mice. This mouse died at 2 mo of age.

On autopsy immediately after death, the spleen was found to be atrophied with a weight of 10 mg, probably reflecting severe wasting, whereas the thymus looked normal with a weight of 29 mg. Calcium deposits were detected in cardiac muscle. No other abnormalities were found on histologic examination in any tissue except for the bones and joints.

Marked proliferation of the synovial tissues was observed as shown in Fig. 2D. Synovial tissues, consisting of the synovial lining cells and fibroblastic cells, extended into the joint space forming villi and covered the surface of the articular cartilage (Fig. 2, D and E). Erosive destruction occurred over the surface of the cartilage due to the invasion of pannus-like granulation tissues that extended even into the bone marrow space (Fig. 2F). The granulation tissues consisted of massive infiltration of inflammatory cells, mostly neutrophils and lymphocytes. Polymorphonuclear leukocytes were also found in the joint space (Fig. 2E). These histopathological findings contrasted markedly with those of the non-Tg healthy joint (Fig. 2C).

CD4-tax-Tg mice, line T5368, also showed similar joint abnormality. Both female and male mice were affected similarly in this line. These mice showed growth retardation, and the spleen was one and one-half to three times heavier than a normal spleen. The joint abnormality became detectable at 6 wk of age, and at 12 wk ~50% of the mice were affected. The histopathology of the joints was similar to that seen in T3376 LTR-tax-Tg mouse. As shown in Fig. 2, G and H, synovial cell overgrowth, bone erosion with granulation tissues, and inflammatory cell infiltration into the granulation tissues were observed in these lesions, although the infiltration of inflammatory cells did not appear as intensive as that seen in T3376 (Fig. 2, D–F). Mice from T5562 and T1887 CD4-tax-Tg line also showed similar histology (data not shown).

These histopathological changes were similar to those found in LTR-pX-Tg mice (13, 14) and in RA in humans. These findings indicate that Tax by itself can cause inflammatory arthropathy resembling RA. In T3376, mild erosive damage could also be found in other joints including the vertebra and tail (data not shown).

**Development of ankylosic polyarthropathy in T4055 line LTR-tax-Tg mice and T5571 LTR-tax-Tg founder mouse**

Descendants from the T4055 LTR-tax-Tg founder mouse showed ankylosic changes of the joints (Table I). Neither swelling nor redness of the joints was observed in this line, although some joint deformities were seen. Extension of the knee joints gradually became difficult with age, starting at 4–6 wk old, and eventually fixed after several months. Incidence of ankylosic joint changes reached to nearly 100% at 10 mo old in C3H/HeN background (Table I). Ankylosic changes could be observed in multiple joints including the vertebral and tail joints in older mice. The histology of 13 mice (5 females and 8 males) was examined macroscopically, and hyperplasia of bony and cartilaginous tissues of the joints was found to be the most characteristic feature of this transgenic line.

As shown in Fig. 4A, the articular cartilage surface of this line of mice was irregular with evidence of fissuring and damage, and erosion of the cartilage and periosteal bone was marked in the joint margins. Secondary ankylosis occurred due to hyperplasia of bony and cartilaginous tissues and fusion of opposing bones (Fig. 4B). Thinning of trabecular with marked osteoclastic activity and woven bone production were observed in the epiphyseal area (Fig. 4C). In this lesion, the bone and marrow cavity were displaced by excessive proliferation of fibrous tissues. Finally, the joint cavity as well as the bone marrow space was completely occupied by newly formed bone (Fig. 4D). Proliferation of the synovial tissues was not obvious. Infiltration of inflammatory cells into synovial tissues was not observed and infiltration into the bone marrow space was found in one case in which there were necrotic changes of the fibula.
Similar histological abnormalities were observed in another LTR-tax-Tg founder mouse, T5571, which developed ankylosis. Swelling of the joints was observed in this mouse, reflecting hyperplasia of boney tissues. As shown Figs. 4, E and F, hyperplasia of cartilaginous tissues and bone were observed both in the ankle joints and in the toe joints. No inflammatory cell infiltration was detected in these joints. Thus, ankylotic joint changes were mainly due to abnormal bone synthesis in the joints. These changes were noticeable only in the joints of the hind legs and forelegs at the beginning, but eventually, most joints including the vertebral and tail joints were affected. These pathological findings were considerably different from those seen in T3376 LTR-tax-, T5368 CD4-tax-, or LTR-pX-Tg mice, in which proliferation of the synovial tissues and infiltration of inflammatory cells were marked.

**FIGURE 3.** Southern blot hybridization of the transgene. DNA from the tails of the T5368 line CD4-tax-Tg, T4055 line LTR-tax-Tg, and LTR-pX-Tg mouse was isolated and analyzed by Southern blot hybridization after digestion with DraI and MboI. A 1.3-kb band is expected for these transgenes (Fig. 1).

**Tax expression in bones of tax-Tg mice**

To know the reason why these tax-Tg mice developed different types of arthropathy, we compared transgene activity in these mice.

**FIGURE 2.** Histopathology of the inflammatory arthropathy in the T3376 line LTR-tax-Tg mouse (D–F) and T5368 line CD4-tax-Tg mouse (B, G, and H). A, Hind legs of control non-Tg mouse (4-mo-old male). B, Hind legs of T5368 (4-mo-old male). Swelling of the ankles and the fingers is remarkable (arrowhead). C, Microscopic observation of the non-Tg ankle joint as a control, magnification, ×33. D, Microscopic observation of the left ankle joint of T3376 line mouse, magnification, ×40. Destruction of joint structure and massive infiltration of inflammatory cells are seen. Proliferating synovial tissue villi with inflammatory cell infiltration are seen to extend into the synovial space. E, Enlargement of the lesion shown in the box in D, magnification, ×200. Extension of fibroblastic cells (large arrowhead) on the bone surface. Thickening of the synovial cell layer (large arrow) is prominent. Exudation of fibrin and fibrinoid substance (small arrowhead) with polymorphnucler leukocytes (small arrow) exists in the synovial space. F, Erosion of bone and formation of pannus, magnification, ×100. The bone is invaded with granulation tissues in which many inflammatory cells consisting of neutrophils and lymphocytes are seen. G, T9048, a female of the T5368 CD4-tax-Tg line, who is 6 mo old. Shown are the toe joints. Note proliferation of synovial tissues and destruction of joint structure with pannus formation, magnification, ×33. H, Enlargement of G, showing the extension of synovial tissues (arrowhead) over the bone surface and infiltration of inflammatory cells are seen, magnification, ×100.
with different phenotypes. Because pups from T3376 and T5571 were not obtained, descendants of T4055 LTR-\textit{tax}-, T5368 CD4-\textit{tax}-, and LTR-pX-Tg mice were compared.

As shown in Fig. 5A, \textit{tax} mRNA was expressed markedly in the thymus of a T5368 line CD4-\textit{tax}-Tg mice in which inflammatory arthropathy developed. Strong expression was also observed in the joints, and weak expression was seen in the spleen. The expression level in the joint was \(1/200\) of that observed in MT-2 cells, as estimated from the intensity of the \textit{tax} bands. On the other hand, \textit{tax} mRNA expression in T4055 LTR-\textit{tax}-Tg mice, in which only ankylosis changes developed, was observed in various tissues including the joints, spleen, thymus, and kidney (Fig. 5B). Interestingly, \textit{tax} mRNA expression in the joints and bones of T4055 LTR-\textit{tax}-Tg mice was 2–4 times lower than that in LTR-pX-Tg mice (Fig. 5C), and \(~20\) times lower than that in T5368 CD4-\textit{tax}-Tg mice (Fig. 5, A and B; Table II), although new bone synthesis was only observed in T4055 line mice. Low levels of \textit{tax} expression in T4055 LTR-\textit{tax}-Tg mice, compared with those in mice that developed inflammatory arthropathy, were confirmed using different strains of mice (Fig. 5D and Table II). Thus, these results suggest that high levels of \textit{tax} expression in the joints induce inflammatory arthropathy and that osteogenesis is not proportionally correlated with the \textit{tax} expression level.

Low levels of autoantibody and inflammatory cytokine production in T4055 line LTR-\textit{tax}-Tg mice

Previously, we showed that various cytokines including IL-1, IL-6, and TNF-\(\alpha\) are induced in the joints of LTR-pX-Tg mice (15). Because these cytokines may be involved in inflammation and bone metabolism, cytokine production in the joints was compared among T4055 line LTR-\textit{tax}-Tg mice, CD4-\textit{tax}-Tg mice, and LTR-pX-Tg mice. Fig. 6 and Table III show the results. mRNA production of IL-1\(\alpha\), IL-1\(\beta\), IL-6, and TNF-\(\alpha\) was markedly enhanced in the joints of both CD4-\textit{tax}-Tg mice (5.1- to 38.7-fold) and LTR-pX-Tg mice (3.2- to 21.8-fold) compared with non-Tg control mice, although the augmentation in LTR-pX-Tg mice appeared less intensive. In contrast, the augmentation of mRNA expression in T4055 line LTR-\textit{tax}-Tg mice was much less (1.4- to 3.8-fold) than those observed in those CD4-\textit{tax}- or LTR-pX-Tg mice with inflammation. In contrast, TGF-\(\beta 1\) (11) expression was strongly induced even in T4055 line LTR-\textit{tax}-Tg mice, and mRNA levels

**Figure 4.** Histopathology of the noninflammatory ankylosis joint changes in T4055 line LTR-\textit{tax}-Tg mice (A–D) and T5571 line LTR-\textit{tax}-Tg founder mouse (E and F). A, In the elbow joint, the cartilage surface degenerates and detaches from the surface (arrowhead), magnification, \(\times 100\). B, Deformity of the toe joints caused by new bone formation. Narrowing of the joint space and fusion of the opposing bones are observed, magnification, \(\times 40\). C, In the digital joints, thinning of the trabecula and hyperplasia of the woven bone are seen. The number of osteoclasts is increased (arrowhead), magnification, \(\times 100\). D, The ankle joint structure is grossly deformed and hardly recognizable because of hyperplasia and bone fusion. The bone marrow space is also filled with bone, magnification, \(\times 40\). E, The ankle joint structure is grossly deformed with hyperplasia of cartilaginous tissues. The joint space is filled with cellular connective tissues, magnification, \(\times 70\). F, In the toe joint, boney bridge is formed between the lateral surface of the bone, magnification, \(\times 40\).
levels were elevated significantly in LTR-pX-Tg mice and T5368 line CD4-tax-Tg mice. Although these Ab levels seemed to be slightly elevated in T4055 line LTR-tax-Tg mice, this elevation was not significant, in clear contrast with the elevation seen in mice with inflammatory arthropathy. Thus, augmented production of inflammatory cytokines and autoantibodies correlates with the development of inflammatory arthropathy, whereas only low levels of cytokines and autoantibodies were detected in mice with ankylosed joints.

### Discussion

In this study, we examined arthritogenic activity of the HTLV-I *tax* gene by producing transgenic mice with two different constructs that carry the *tax* gene as a sole functional transgene. We produced seven and nine transgenic founder mice using LTR-tax and CD4-tax transgene, respectively, and found that one of the LTR-tax founder mice and five CD4-tax-Tg lines developed chronic inflammatory arthropathy, while two of LTR-tax-Tg lines developed ankylotic changes of the joints. Histological examination of the lesion that developed in T3376 LTR-tax-Tg founder mouse revealed that both bone and cartilage were destroyed by invasion of hypertrophic synovial tissues and inflammatory cells which infiltrated into these tissues forming pannus. We found that CD4-tax-Tg mice, including the T1887, T5368 and T5562 lines, also developed similar inflammatory arthropathy. The histopathology seen in these mice was very similar to that found in LTR-pX-Tg mice (14). Involvement of LTR in the pathogenesis is unlikely, since no open reading frame is known in that region. Furthermore, development of arthritis both in LTR-tax-Tg and in CD4-tax-Tg mice indicates that the promoter/enhancer region is not directly involved in the pathogenesis. The possibility that insertional mutation by the transgene causes arthritis also seems unlikely, because so far five independent transgenic mice including pX-Tg, and T1887, T3376, T5368, and T5562 tax-Tg mice with three different constructs developed similar inflammatory arthropathy. Thus, these data indicate that the *tax* gene is arthritogenic and suggest that this gene is the causative gene for the inflammatory arthropathy that developed in LTR-pX-Tg mice.

Interestingly, we found that noninflammatory ankylosiac arthropathy also developed in two LTR-tax-Tg mice. Joint deformity caused by hyperostogenesia was the major change seen in these mice, and neither synovial tissue hyperplasia nor inflammatory cell infiltration was observed in the affected joints. These observations were markedly different from those seen in LTR-pX-Tg, T3376 LTR-tax-Tg and T5368 CD4-tax-Tg mice. Involvement of insertional mutations at the integration site in the ankylotic changes seems unlikely, since two independent founder mice were affected.

#### Table II. *tax* expressions in joints among different *tax*-transgenic strains

<table>
<thead>
<tr>
<th>Transgenic Strain</th>
<th><em>tax</em> Expression (×10^3)<em>a</em></th>
<th>Inflammation</th>
<th>Ankylosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTR-tax</td>
<td>2.8 ± 0.1</td>
<td>0</td>
<td>45.5</td>
</tr>
<tr>
<td>LTR-pX</td>
<td>10.1 ± 4.0*</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>CD4-tax</td>
<td>50.1 ± 12.5*</td>
<td>43.6</td>
<td>0</td>
</tr>
</tbody>
</table>

* a Means and SD are shown. Statistical analysis was performed to LTR-tax group: *p < 0.05 (F test), †p < 0.01 (F test).

*tax* expression levels are compared with that of MT-2 (n = 3, except CD4-tax: n = 4). All the mice analyzed already developed symptoms.

The numbers of mice examined were 101, LTR-tax, 261, LTR-pX and 34, CD4-tax. The mice were inspected at 3 mo of age.
Thus, the results suggest that Tax can cause at least two different types of arthropathy in mice.

To elucidate mechanisms by which two different types of disease develop in tax transgenic mice, we examined the expression of inflammatory cytokines and autoantibody levels in the serum. We found that both cytokine and autoantibody levels, except for the TGF-β1 level, were much lower in T4055 line LTR-tax-Tg mice with joint ankylosis than in LTR-pX-Tg or T5368 line CD4-tax-Tg mice that develop inflammatory arthropathy. In this connection, we demonstrated that the tax mRNA expression level in joints and in bone from T4055 line mice was lower than that in LTR-pX-Tg-Tg mice. Thus, these observations suggested that a high level of tax expression in the joints causes inflammatory cytokine production resulting in the induction of inflammatory arthropathy (15), whereas a low level of tax expression only induces osteogenesis in the joints. In this context, it is worth noting that TGF-β1 expression was markedly elevated in the joints of T4055 line mice in contrast to the moderate activation of IL-1, IL-6, and TNF-α expression in the same joints. Because TGF-β1 is a potent inducer of osteogenesis (30), the activation of this gene could be involved in the enhanced osteogenesis in T4055 line mice.

In a previous paper, Ruddle et al. (31) reported that transgenic mice carrying the tax gene exhibit skeletal alterations resembling Paget’s disease. High bone turnover caused by the increase of both osteoblast and osteoclast is characteristic of their transgenic mice, although they did not describe the development of arthropathy in these mice. In contrast, the number of osteoclasts was within the normal range in T3376, T5368, and LTR-pX-Tg mice that developed RA-like arthritis, and no obvious remodeling of the bone could be observed. Although some activation of osteoclast was observed in T4055 line mice, the activation was not strong and mainly hyperosteo genesis was observed in these mice. Therefore, the histopathological appearance of these mice differs considerably from those reported previously (31). These observations indicate that Tax can cause a broad spectrum of abnormalities in the joints.

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FIGURE 6. Cytokine gene expression in the joints of tax transgenic mice. Poly(A)+ RNA was prepared from the joints, and IL-1α, IL-1β, IL-6, TNF-α, and TGF-β1 mRNA levels were compared among a LTR-pX-Tg mouse (a 3-mo-old male), a CD4-tax-Tg mouse (a male from the line T5368 at 3 mo old), and a LTR-tax-Tg mouse (a male from the line T4055 at 3 mo old) by Northern blot hybridization. A nontransgenic mouse of the same sex and age was used as a control. The same filter was used throughout the experiments, and a β-actin band was used to verify the RNA load.

FIGURE 7. Ig and autoantibody levels in the serum of LTR-pX-, CD4-tax-, and LTR-tax-Tg mice. Serum Ig and autoantibody levels were measured in arthritic LTR-pX-Tg mice, CD4-tax-Tg mice (line 5368), ankylo tic LTR-tax-Tg mice (line T4055), and non-Tg control mice. A. Serum total IgG; B. serum total IgM; C. anti-bovine-IIC Ab of the IgG class; D. rheumatoid factor of the IgG class. Means and SD are shown. Statistical analyses were performed to non-Tg group. ‡, p < 0.001; †, p < 0.01 (by F test); and §, p < 0.05 (by Mann-Whitney U test).
Ref. 13). Therefore, expression levels of the tax gene in lymphoid tissues may not be the only factor that controls autoimmune development. Further study is in progress to elucidate the pathogenesis of inflammatory arthropathy and ankylosis using tax transgenic mice with different promoters.

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