mitotic kinase activity 5 μl of p13^{suc1} or mock beads (*17*) were incubated at 4°C for 1 hour in the presence of 400 μg of WCE diluted in 2× bead buffer [bead buffer: 50 mM tris-Cl, pH 7.4, 5 mM NaF, 250 mM NaCl, 5 mM EDTA, 5 mM EGTA, 0.1% NP-40, 100 μM benzamidine, leupeptin (10 μg/ml), and aprotinin (10 μg/ml)]. Beads were washed twice with bead buffer and once with kinase buffer (25 mM MOPS, pH 7.2, 60 mM β-glycerophosphate, 5 mM EGTA, 15 mM $MgCl_2$, 1 mM dithiotheitol, 100 μM benzamidine) and incubated in 30 μl of kinase buffer that contained 1.6 μM adenosine triphosphate, 0.1 μM ^{32}P[γATP], 10 to 20 ng of Oct1, and 2.5 μg of histone H1. Phosphorylation of Oct1 was carried out for 10 min at 30°C.

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Induction of Inflammatory Arthropathy Resembling Rheumatoid Arthritis in Mice Transgenic for HTLV-I

YOICHIRO IWAKURA,* MARIKO TOSU, EMI YOSHIDA, MASAFUMI TAKIGUCHI, KAZUTO SATO,† ISAO KITAJIMA,† KUSUKI NISHIOKA,† KAZUHIKO YAMAMOTO, TOSHIO TAKEDA, MASAKAZU HATANAKA, HIROAKI YAMAMOTO, TOYOZO SEKIGUCHI

Human T cell leukemia virus type-I (HTLV-I) is the etiologic agent of adult T cell leukemia and has also been suggested to be involved in other diseases such as chronic arthritis or myelopathy. To elucidate pathological roles of the virus in disease, transgenic mice were produced that carry the HTLV-I genome. At 2 to 3 months of age, many of the mice developed chronic arthritis resembling rheumatoid arthritis. Synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissues were marked. These observations suggest a possibility that HTLV-I is one of the etiologic agents of chronic arthritis in humans.

VARIOUS ETIOLOGIC AGENTS INcluding viruses (*1, 2*), bacteria (*3–5*), and mycoplasmas (*6*) have been suggested as causes of rheumatoid arthritis in humans. However, so far no convincing evidence has been presented for a role of these agents in the disease. A high incidence of chronic arthritis has been reported in patients with HTLV-I–associated myelopathy, suggesting a possible involvement of HTLV-I in the disease (7). However, pathological roles of the virus, if any, remain obscure, because so far arthritogenic activity of the virus has not been shown. To examine the pathological effects of the virus on an animal, we produced lines of transgenic mice carrying the HTLV-I genome.

We constructed plasmid pHLX-I (8.8 kb), consisting of the 5′ long terminal repeat (LTR) 3′ end of *pol* [192 nucleotides (nts)]-*env-pX*-3′ LTR of HTLV-I, by replacing the Pvu II–Hind III fragment of pKCROHS (*8*) with the Sma I–Hind III fragment from the 5′ LTR clone of HTLV-I (pU3RU5) (Fig. 1B). We produced transgenic mice by injecting the Eco RI fragment (5.3 kb) containing the whole HTLV-I–derived sequence into fertilized mouse ova [(C3H/HeN × C57Bl/6J)F_2]. Both transgenic and nontransgenic siblings were kept in the same cages, and we checked pathological changes without knowing the genotype. Mice were cared for according to institutional guidelines and were kept in laminar-flow racks.

From a total of 960 ova injected, seven transgenic mice were obtained, and two mouse lines (T647 and T649) were established from these mice. Southern blot hybridization analysis of the tail DNA revealed that five to ten copies of the injected DNA were integrated intact into a single site of the host chromosome in a tandem fashion in both the T647 and T649 lines (*9*). Messenger RNA from the transgene could be detected in both line T647 and T649, but the level was more than 20 times higher in those mice from line T647 than in those from T649. Tissue specificity of the expression was rather broad; mRNA was strongly expressed in the brain, salivary gland, and joints and less intensely in various tissues, including the spleen, lung, eyeballs, muscle, and skin (Fig. 1A). Approximately equal amounts of mRNAs specific to the Env and pX proteins were detected in these tissues. The expression level of mRNA in the joints was approximately 1/2000 of that in MT-2 cells.

Swelling of the ankle with redness or swelling of the footpad near the ankle or all three symptoms were detected in 48 mice out of 141 transgenic T647 mice (Figs. 2 and 3A) and in 12 mice out of 126 transgenic T649 mice. In contrast, only one mouse out of 190 nontransgenic siblings demonstrated these changes, and none in unrelated transgenic mice that carry the human hepatitis B viral genome (104 mice) or the mouse interferon β gene (78 mice). The abnormality began to occur in female mice at age 2 to 3 months, and the proportion of affected mice increased thereafter until one year of age. At one year of age, almost half of the mice were affected. Onset in male mice was delayed 5 to 10 months, and the incidence was lower than that in female mice (10/47 and 38/94, respectively, in line T647 and 5/70 versus 7/56, respectively, in line T649). These abnormalities usually occurred in multiple ankle joints of a mouse. In some cases (5/141), swelling regressed after 1 to 4 months. The amounts of the mRNA specific to the transgene in the joints from affected mice were five to ten times higher than in those from apparently normal mice.

Joints from 15 affected transgenic mice were examined microscopically, and 12 of them were abnormal, whereas joints from 13 apparently normal transgenic mice showed no obvious changes except for slight degenerative changes of cartilage (4/13) and slight inflammatory cell infiltration in the synovial tissues (5/13). No abnormal changes were detected in joints from ten control nontransgenic mice. Histological observations of the affected joints are shown in Fig. 3. Erosion of the synovial bones and cartilage was marked in the ankle joint, and these tissues were replaced with pannus-like granulation tissues, which were composed of fibroblasts and small blood vessels accompanied by infiltration of lymphocytes, neutrophils, and macrophages (Fig. 3, B and C). Similar pathological changes were also observed in the joints of the toes (one in the four affected mice) and the knees (two in the four affected mice). In seven cases in the 13 affected mice, infiltration of inflammatory cells was found in the surrounding dermis, in the subcutaneous cells, and around the

Y. Iwakura, M. Tosu, E. Yoshida, M. Takiguchi, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan.
K. Sato, I. Kitajima, K. Nishioka, Institute of Rheumatology, Tokyo Women's Medical College, Shinjuku-ku, Tokyo 163, Japan.
K. Yamamoto, Faculty of Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.
T. Takeda, Chest Disease Research Institute, Kyoto University, Sakyo-ku, Kyoto 606, Japan.
M. Hatanaka, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606, Japan.
H. Yamamoto and T. Sekiguchi, Kohno Clinical Medicine Research Institute, 3-3-7 Kitashinagawa, Shinagawa-ku, Tokyo 140, Japan.

*To whom correspondence should be addressed.
†Present address: Rheumatology Division, Institute of Medical Science, School of Medicine, St. Marriana University, Miyamac-ku, Kawasaki 213, Japan.

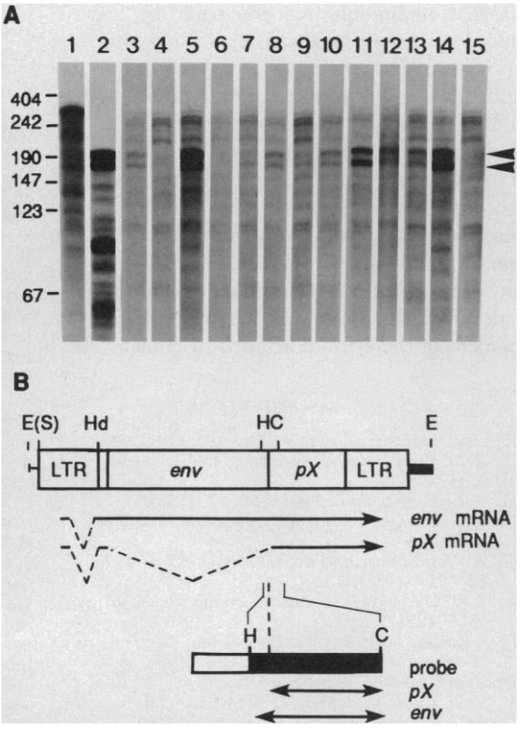

Fig. 1. Tissue specificity of transgene expression. (**A**) Messenger RNAs specific to the transgene were detected by ribonuclease (RNase) protection assay (*29*). Total RNA [40 μg, except lane 2 (0.1 μg) and lane 10 (15 μg)] was obtained (*30*) from different tissues of a T647 F_1 transgenic mouse (9 weeks old, female) with affected joints. The RNA from the joints was prepared from the knees, ankles, and the digits from which the skin and the muscle were removed. An antisense RNA probe (297 nt) was made with SP6 RNA polymerase with the Hpa I–Cla I fragment (191 nt) from pHLX-I that had been subcloned into pGEM-4 (Promega Biotec, Madison, Wisconsin) as a template. Hybridization was carried out at 45°C overnight, followed by RNase A (40 μg/ml) with RNase T_1 (2 μg/ml) at 30°C for 1 hour (*31*). Autoradiograms were exposed for 7 days, except for lane 2 (3 hours). The band at 191 nt represents *env*-mRNA and that at 181 nt represents *pX*-mRNA (arrowheads). Lane 1, probe; lane 2, HTLV-I–producing T cells (MT-2) as the positive control. Lanes 3 through 14 are samples from a transgenic mouse. Lane 3, spleen; lane 4, thymus; lane 5, brain; lane 6, liver; lane 7, kidney; lane 8, lung; lane 9, heart; lane 10, eyeball; lane 11, salivary gland; lane 12, muscle; lane 13, skin; lane 14, joints; and lane 15, joints from a nontransgenic mouse. Molecular size standards are shown at left in nucleotides. (**B**) Structure of the DNA injected and the RNA protected from RNase treatment. Box (top): HTLV-I sequence (4.8 kb); thin line, simian virus 40 sequence (70 nt); thick line, cellular sequence (400 nt). E, Eco RI; S, Sma I; Hd, Hind III; H, Hpa I; C, Cla I. The probe is 297 nt; *pX* is 181 nt; *env* is 191 nt. (Middle) Solid lines indicate mRNA; dashed lines indicate regions of splicing. (Bottom) Dark box indicates HTLV-I sequence; open box indicates pGEM-4 sequence.

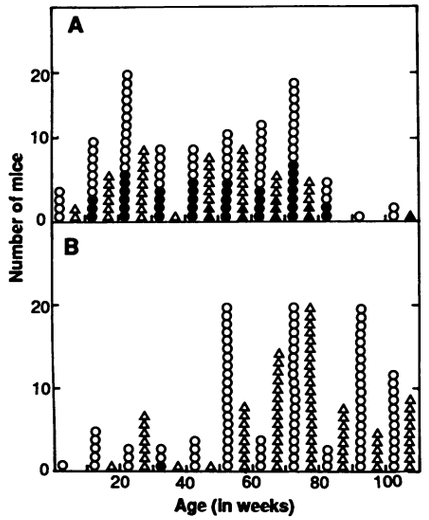

Fig. 2. Incidence of arthritis in transgenic mice of line T647. All the mice in the mouse colony were categorized by age, and lesions of the ankle joints in each category were examined by eye. The severity was graded on a scale of 0 to 2 for each paw, based on the degree of redness and swelling, and mice with lesions of grade 1 or more in at least one paw are indicated by filled symbols. Females are indicated by circles; males by triangles. Control mice were the littermates of transgenic mice. (**A**) Transgenic mice. There were 94 females and 47 males. (**B**) Control mice. There were 103 females and 87 males.

blood vessels. In these affected joints, synovial proliferation associated with stratification of synovial lining cells and degeneration of cartilage was frequently observed (11/13) (Fig. 3D). These histopathological changes closely resembled rheumatoid arthritis in humans (*10*).

The thymuses of these transgenic mice frequently became atrophied, and the proliferative response of the T cells to concanavalin A was reduced (*11*). In these transgenic mice, no antibodies to HTLV-I were detected by Western blot analysis. One of the six mice examined produced rheumatoid factor at a low level, about 1/20 that of control autoimmune MRL-*lpr/lpr* mice (*12*), but no correlation with the symptom was observed. Antibodies to both double- and single-stranded DNA of the immunoglobulin G class were detected in two mice in that group (the level about 1/25 that in MRL-*lpr/lpr* mice). Severe arthritis with pannus formation was noted in these two mice.

We have demonstrated the arthritogenic capacity of HTLV-I in mice. This observation suggests the possibility that HTLV-I is involved in chronic arthritis in humans. In fact, association of HTLV-I with rheumatoid arthritis was suggested in patients with adult T cell leukemia (ATL) and HTLV-I–associated myelopathy (*7, 13*). Although the pathology remains to be elucidated, two other related retroviruses have also been suggested to be involved in chronic arthritis: caprine arthritis-encephalitis virus in goats (*14*) and human immunodeficiency virus (*15, 16*).

As further evidence of the roles of the virus in the pathology of arthritis, the mRNA of Tax, a trans-acting transcriptional activator coded in the *pX* region of the virus (*17*), is markedly expressed at the joints. Because Tax is known to be a potent inducer of various cellular genes (*18, 19*), it is possible that some cytokines that induce inflammation or bone destruction (*20*) are themselves induced at the joints. In support of this notion, we noticed that high levels of interleukin-1α mRNA are expressed in the joints of transgenic mice (*21*). Interleukin-1 is also reported to be detected in peripheral T cells (*22*) and synovial cells (*23*) from individuals with ATL. Promotion of the growth of synovial cells in transgenic mice may also reflect the effects of these cytokines or the direct cell

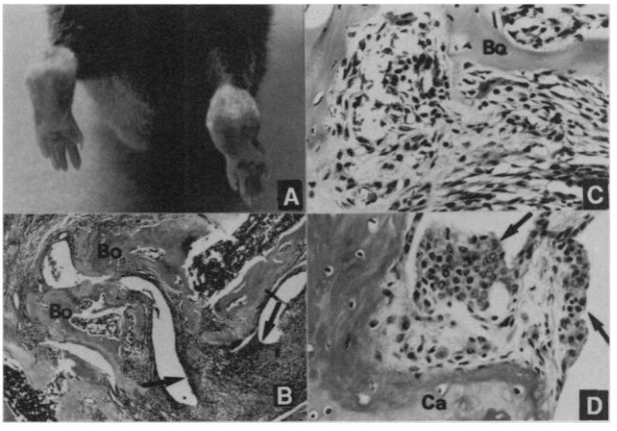

Fig. 3. Histology of the joints. (**A**) The ankles and the toes of an affected mouse (T3085, a female F_2 mouse from line T647, 42 weeks old). Swelling of the joints was marked. (**B**) Histopathology of the ankle joint (×65), showing erosive destruction of the bone and pannus formation (arrows). Infiltration of the inflammatory cells is noticeable. (**C**) Invasion of fibroblastic cells and inflammatory cells consisting of neutrophils and lymphocytes (×260). (**D**) Proliferation of synovial stromal cells (arrows) accompanied by mild degenerative change in the cartilage (T5533, a female, 30 weeks old) (×260). Hematoxylin and eosin staining in (B) through (D). Bo, bone; Ca, cartilage.

growth–promoting activity of Tax (*24–26*). However, it should be noted that no Tax or Env proteins could be detected in the joints by both immunoblotting and immunohistochemical techniques. This is probably due to low amounts of the proteins, because the mRNA level of these proteins was low even in the affected joints, being approximately 1/2000 of that detected in MT-2 cells. Nonetheless, the observation that the amounts of HTLV-I mRNA expression in affected joints were five to ten times higher than in apparently normal mice suggests a correlation between the RNA expression and the joint disease. Probably, small amounts of Tax are enough to exert an influence on cells at the joint.

Another possible explanation for the induction of inflammatory arthropathy is an immunological disturbance by the transgene. The observation that some of the mice had antibodies to immunoglobulins and DNAs suggests autoimmune-like complications in these mice. Although exocrine pathology resembling Sjögren's syndrome, a presumed autoimmune disease, has been found in *tax* transgenic mice (*27*), our transgenic mice showed no abnormalities in the salivary and lachrymal glands. The possibility that an immune reaction could be induced against synovial cells expressing viral antigens or antigens cross-reactive with viral antigens seems less likely; no antibodies to HTLV-I antigens have been detected in these transgenic mice, and no cross-reactivity of the Env and pX antigens with rheumatoid synovium is known. Gag does cross-react with rheumatoid synovium, but it is not carried by these transgenic mice (28). In any event, these transgenic mice should provide a useful model to investigate the development of rheumatoid arthritis in humans.

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Identification of a Site in Glutamate Receptor Subunits That Controls Calcium Permeability

RICHARD I. HUME, RAYMOND DINGLEDINE, STEPHEN F. HEINEMANN*

The neurotransmitter glutamate mediates excitatory synaptic transmission throughout the brain. A family of genes encoding subunits of the non–*N*-methyl-D-aspartate (non-NMDA) type of glutamate receptor has been cloned. Some combinations of these subunits assemble into receptors with a substantial permeability to calcium, whereas others do not. To investigate the structural features that control ion permeation through these ligand-gated channels, mutant receptor subunits with single–amino acid changes were constructed. Mutation of a certain amino acid that results in a net charge change (from glutamine to arginine or vice versa) alters both the current-voltage relation and the calcium permeability of non-NMDA receptors. A site has thus been identified that regulates the permeation properties of these glutamate receptors.

INFLUX OF CA THROUGH GLUTAMATE receptors (GluR) is thought to play a key role in long-term potentiation, excitotoxic neuron damage, and epilepsy (*1*). In many neurons, non-NMDA glutamate receptors have low permeability to Ca^{2+} (*2*). However, in a few types of neurons, Ca^{2+} influx through non-NMDA receptor channels is more pronounced (*3*). The four subunits of the first family of non-NMDA glutamate receptors to be cloned (*4–6*), named GluR1, GluR2, GluR3, and GluR4 (*7*), are similar to one another in amino acid sequence. When expressed in *Xenopus* oocytes alone or in combination, GluR1 and GluR3 form receptors that have a substantial Ca^{2+} permeability, but when GluR2 is coexpressed with either GluR1 or GluR3, the receptor channels have little or no Ca^{2+} permeability (*8*). The current-voltage (*I-V*) relations of these subunit combinations are also distinct. GluR1 or GluR3 receptor channels display a strongly inwardly rectifying *I-V* relation, whereas these subunits coassembled with GluR2 have a linear or outwardly rectifying *I-V* relation (*5, 6*).

To determine the regions of these proteins that are responsible for the permeation properties of the channels, we first examined the functional properties of subunit chimeras. These experiments indicated that a region extending from the middle of the NH_2-terminal extracellular domain through the third putative transmembrane segment determined the shape of the *I-V* curve (*9*). In this region, the amino acid sequence similarities of GluR1, GluR2, and GluR3 are high. We searched for positions in this region in which the corresponding amino acid is the same in GluR1 and GluR3 but different in GluR2 and found one charge substitution in a region of otherwise identical amino acid sequence (Fig. 1A). In this region, a glutamine is present in GluR1 and GluR3, the two subunits with rectifying *I-V* relations, whereas arginine resides in the equivalent position of GluR2, the subunit that can combine to form receptors with linear *I-V* relations. This charge difference is near one end of a sequence that has been postulated to form the second membrane-spanning domain (*6*) or an extracellular loop

R. I. Hume, Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037, and Department of Biology, University of Michigan, Ann Arbor, MI 48109.
R. Dingledine, Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037, and Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599.
S. F. Heinemann, Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037.

*To whom correspondence should be addressed.