

# Articular tissues expressing the *env-pX* transgene are required for generation of arthritogenic T cells in human T cell leukemia virus type I transgenic rats

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This work was supported by grants from the Ministry of Education, Science, Technology, Sports, and Culture, and the Ministry of Health, Labour and Welfare, of Japan, and from Takeda Science Foundation.

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**Key words:** Adoptive transfer, animal model, arthritis.

## ABSTRACT

**Objective.** Human T cell leukemia virus type I *env-pX* transgenic rats (*env-pX* rats) were used to investigate the pathogenesis of arthritis.

**Methods.** Phenotype of cells infiltrated into arthritic joints in *env-pX* rats was analyzed using flow cytometry and cell-transfer experiments were done using *env-pX* and wild-type WKAH rats.

**Results.** The majority of T cells infiltrated into arthritic joints in *env-pX* rats exhibited a CD4 and activated phenotype. Transfer of these T cells into articular space in wild-type WKAH rats succeeded to induce arthritis similarly seen in *env-pX* rats. However, injection of the cells into sites other than joints did not induce inflammation. Transfer of in vitro-stimulated lymph node cells from disease-free *env-pX* rats into articular space did not induce arthritis in wild-type WKAH rats.

**Conclusion.** These findings suggest that articular tissues carrying the *env-pX* transgene are required for generation of arthritogenic T cells in *env-pX* rats. However, the constitutive antigens other than the transgene products are recognized as immunological targets by the arthritogenic T cells in the advanced arthritic joints. Molecules expressed specifically in articular tissues may be needed to maintain the inflammatory cell infiltration.

## Introduction

Human T cell leukemia virus type I (HTLV-I) is the pathogenic agent of adult T cell leukemia and is also associated with a number of inflammatory diseases, including myelopathy, uveitis, and probably arthropathy (1). Since p40tax (Tax) encoded by HTLV-I *env-pX* gene modulates expression and function of host molecules such as cytokines, cytokine receptors, growth factors, transcription factors, and cell cycle-related molecules, it is considered that Tax plays the major pathogenic roles in HTLV-I-associated diseases. To investigate the roles of Tax *in vivo*, we established several transgenic rat models (2-4). Among them, WKAH rats carrying the *env-pX* gene developed chronic destructive arthritis similar to rheumatoid arthritis (RA), necro-

tizing arteritis resembling polyarteritis nodosa, and other connective tissue diseases (3). The *env-pX* transgene was expressed constitutively without tissue or cell-specificity in these rats (*env-pX* rats) under control of HTLV-I long terminal repeat promoter. Since autoantibodies, including rheumatoid factors, were present in sera, it is believed that autoimmune mechanisms are involved in the pathogenesis of diseases occur in *env-pX* rats. Prior to development of diseases, progenitors of B cells increased in the bone marrow (5). Peripheral T cells were pre-activated to express intercellular adhesion molecule-1 (ICAM-1) and CD80/86, and showed a hyper-response against several mitogenic stimuli *in vitro* (6). Loss of function of CD25<sup>+</sup> CD4<sup>+</sup> immunoregulatory T cells was evident before these rats developed autoimmune diseases (7, 8). It is thought that the abnormalities in lymphocytes were caused by the *env-pX* transgene expressed in these cells. Interestingly, although vasculitis occurred in irradiated wild-type WKAH rats reconstituted by *env-pX* spleen cells, arthritis did not develop (9). Furthermore, vasculitis, not arthritis, occurred in wild-type WKAH rats transplanted thymus framework from *env-pX* rats (9). These findings clearly indicate that diverse mechanisms are implicated in the development of arthritis and vasculitis. In the present study, we examined arthritogenic T cells in *env-pX* rats and discussed the pathogenesis of arthritis.

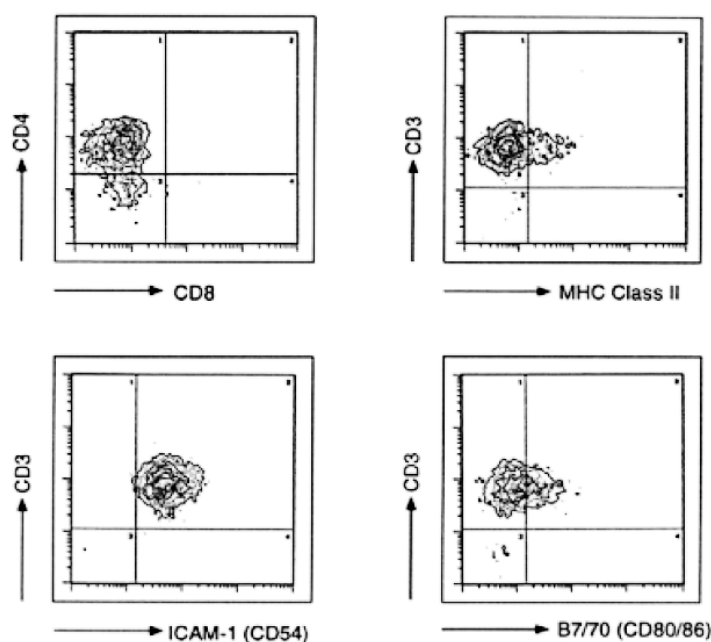
## Materials and methods

### Rats

Inbred WKAH rats and WKAH rats bearing the *env-pX* gene (*env-pX* rats) (3) were maintained at the Institute for Animal Experimentation, Hokkaido University Graduate School of Medicine. Experiments using animals were done in accordance with the Guide for the Care and Use of Laboratory Animals in Hokkaido University Graduate School of Medicine.

### Separation of mononuclear cells from arthritic joints and lymph nodes

Swollen tissues around the ankle joints of *env-pX* rats were excised into small



**Fig. 1.** Flow cytometry for IMC from arthritic joints of env-pX rats (2 months old) is shown. This assay was done using FACScan (Becton Dickinson, Franklin Lakes, NJ). IMC were separated from at least 3 rats and experiments were repeated independently. Representative results are shown.

**Table I.** Diameter of ankle joints 1 week after intra-articular cell-transfer.

Cells injected into rt ankles	Donor rat	Diameter of ankle joints		p-value
		rt	lt (PBS injected)	
Nonstimulated LNC	WKAH	8.03 ± 0.05	7.95 ± 0.02	0.58
Con A-stimulated LNC	WKAH	7.68 ± 0.01	7.63 ± 0.01	0.50
Nonstimulated LNC	env-pX	7.72 ± 0.04	7.60 ± 0.05	0.11
Con A-stimulated LNC	env-pX	7.68 ± 0.07	7.53 ± 0.05	0.18
Infiltrating cells from arthritic joints	env-pX	9.38 ± 3.65	7.33 ± 0.35	0.01

LNC: lymph node cells; rt: right; lt: left.

pieces and then stirred in RPMI 1640 medium containing 1% collagenase (Wako, Tokyo, Japan) and 60 units/ml heparin for 1 hour at 37°C. After removal of tissue-debris by filtration, mononuclear cells were separated using Lympholyte Rat (Cedarlane, Ontario, Canada). Mononuclear cells were also separated from cervical lymph nodes of WKAH and env-pX rats using Lympholyte Rat.

#### Flow cytometry

Monoclonal antibodies used were anti-rat CD3, G4.18 (Pharmingen, San Diego, CA); CD4, RTH-7 (10); CD8, 10B5 (10); ICAM-1 (CD54), 1A29 (10); and MHC class II, ISCR3 (6). To detect rat B7/70 (CD80/86), the recom-

binant chimera protein of mouse CTLA4 and human IgG (CTLA4-Ig) (11), obtained from Dr. M. Azuma (Tokyo Medical and Dental University, Tokyo, Japan), was used.

#### Stimulation of mononuclear cells

For stimulation *in vitro*, mononuclear cells were incubated with 5 µg/ml of concanavalin A (Con A) (Sigma-Aldrich, St. Louis, MO) for 48 hours at 37°C.

#### Injection of cells into articular space

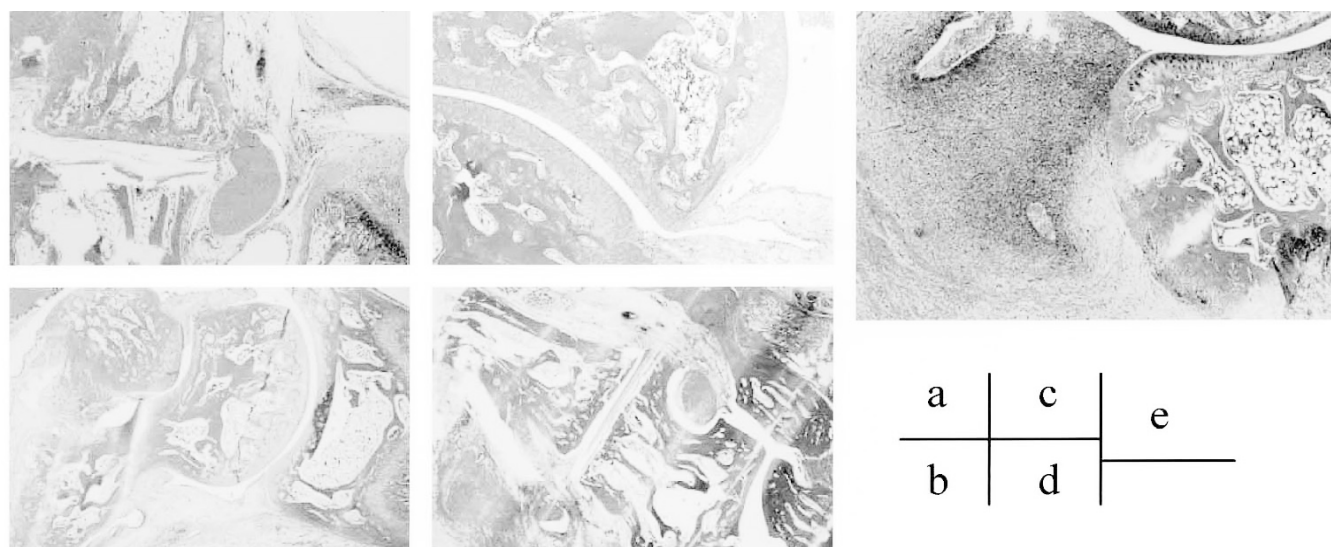
Stimulated or unstimulated mononuclear cells ( $1 \times 10^6/100 \mu\text{l}$ ) were injected into articular space of right ankle joints of wild-type WKAH rats. For control experiments, PBS (100 µl) was injected

into the opposite site. Diameter of joints was measured daily. Seven days after injection, rats were killed for processing of tissue sections.

#### Results

Infiltrating mononuclear cells (IMC) separated from arthritic joints of env-pX rats were analyzed using flow cytometry (Fig. 1). Most cells were positive for CD3, indicating that the composition of IMC was mostly T cells. Immunohistochemistry showed that the major mononuclear cells infiltrated into affected organs in env-pX rats were T cells and macrophages (data not shown). Thus, it is considered that macrophages were depleted during the procedure of IMC separation. On the other hand, a significant infiltration of B cells into affected tissues was not evident in env-pX rats. Interestingly, the majority of T cells in the IMC exhibited a CD4 phenotype, corresponding to the finding that CD4<sup>+</sup> T cells dominantly infiltrated into synovial tissues in RA patients (12). In addition, most IMC separated from env-pX arthritic joints were positive for ICAM-1 (CD54) and a part expressed MHC class II and B7/70 (CD80/86). These molecules, which are expressed on T cells by various stimuli (6), implied an activated phenotype of T cells.

Diameters of right ankle joints, which had been given intra-articular injection of cells, were compared with those of left ankle joints inoculated with PBS (Table I). When IMC from arthritic joints of env-pX rats were injected into WKAH ankles, joints significantly swelled 1 week later. No significant change was observed when lymph node cells (LNC) from env-pX rats without arthritis or from WKAH rats were injected. Significant swelling of joints was not induced even by transfer of LNC, which had been stimulated by Con A. Histological examinations revealed that arthritis similar to env-pX rats occurred in WKAH rats, which had been given intra-articular injection of env-pX IMC (Fig. 2). Infiltration of mononuclear cells corresponding to the transferred T cells and proliferation of synovial tissues were evident in the



**Fig. 2.** Histological findings on right ankle joints of wild-type WKAH rats (6 weeks old), which were given intra-articular injection of cells ( $1 \times 10^6/100 \mu\text{l}$ ) 1 week before, are presented. The cells injected were nonstimulated LNC from WKAH rats (a), Con A-stimulated ( $5 \mu\text{g/ml}$ , 48 hours) LNC from WKAH rats (b), nonstimulated LNC from env-pX rats without arthritis (c), Con A-stimulated ( $5 \mu\text{g/ml}$ , 48 hours) LNC from env-pX rats without arthritis (d), and IMC from arthritic joints of env-pX rats (e). In each group, at least 5 rats were examined. Donor rats were used at 2 months old of age. Representative photos are shown.

arthritic joints. In rats of other groups, most of the transferred cells disappeared from the joints 1 week after transfer. Injection of IMC from arthritic joints of env-pX rats into WKAH subcutis or muscles did not induce inflammation at the sites (data not shown).

### Discussion

During T cell differentiation, autoreactive lymphocytes are eliminated in the thymus (13). In this negative selection, framework of thymic medulla plays a critical role in presenting self-antigens. Recent studies demonstrate that the *aire* gene is implicated in the self-presentation in thymic medulla, and that deficiency of the gene results in development of syndromes, including autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy (14, 15). However, it remains unknown how autoreactive T cells are generated in common autoimmune diseases. Our previous cell-transfer experiments showed that necrotizing arteritis, similarly seen in env-pX rats, occurred in irradiated wild-type WKAH rats reconstituted by env-pX spleen cells (9). By contrast, arthritis never developed in wild-type recipients. Furthermore, vasculitis but not arthritis appeared in wild-type WKAH rats transplanted thymus framework from env-pX rats

(9). These findings suggest that thymus framework carrying the *env-pX* transgene is critically involved in the generation of T cells autoreactive to self-vasculature, and that arthritis occurred through completely different mechanisms from vasculitis in env-pX rats. In order to investigate the pathogenesis of arthritis, we examined a phenotype of cells infiltrated into arthritic joints in env-pX rats, and subsequently performed a set of cell-transfer experiments.

The majority of T cells infiltrated into arthritic joints in env-pX rats exhibited a CD4 and activated phenotype, corresponding to findings in RA patients (12). Transfer of these T cells into articular space in wild-type WKAH rats succeeded to induce arthritis similarly seen in env-pX rats. Although we cannot rule out that the *env-pX* gene products are recognized as immunological targets in the early phase of arthritis, these findings indicate that the transgene in articular tissues are not required for the progression of arthritis. On the other hand, since transfer of IMC from env-pX arthritic joints did not induce inflammation into other sites such as subcutis, it is considered that molecules expressed specifically in articular tissues may be needed to maintain the inflammatory cell infiltration.

We recently suggested that IL-6 secreted from synovial cells expressing the *env-pX* transgene promoted chronic arthritis in env-pX rats (16).

One of the most important findings in this study is that transfer of *in vitro*-stimulated LNC from disease-free env-pX rats into articular space did not induce arthritis in wild-type WKAH rats. These findings suggest that articular tissues are required for the generation of arthritogenic T cells in env-pX rats. Since Tax encoded by the *env-pX* transgene functions as a transactivator for various genes (1), activated articular tissues, such as cytokine-producing synovial cells, may be associated in the generation of arthritogenic T cells.

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