The point mutation of tyrosine 759 of the IL-6 family cytokine receptor gp130 synergizes with HTLV-1 pX in promoting rheumatoid arthritis-like arthritis

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Abstract

Rheumatoid arthritis (RA) is a polygenic autoimmune disease. The autoimmunity develops from synergistic actions of genetic and environmental factors. We generated a double-mutant mouse by crossing two murine models of RA, a gp130 mutant knock-in mouse (gp130F759/F759) and an HTLV-1 pX transgenic mouse (pX-Tg), in a C57BL/6 background, which is resistant to arthritis. The mice spontaneously developed severe arthritis with a much earlier onset than the gp130F759/F759 mice and with a much higher incidence than did the pX-Tg mice. The symptoms of gp130F759/F759 mice, including lymphadenopathy, splenomegaly, hyper-γ-globulinemia, autoantibody production, increases in memory/activated T cells and granulocytes in the peripheral lymphoid organs, and a decrease in the class II MHCbright CD11c+ population, were augmented in the double mutants. Marked reductions in incidence, severity and immunological abnormalities were seen in the triple mutant, IL-6−/−/gp130F759/F759/pX-Tg, indicating that the arthritis in the double mutant is IL-6 dependent. gp130F759/F759/pX-Tg is a unique mouse model for RA.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by the progressive chronic inflammation of multiple joints, which leads to their destruction, thus disabling the patients. The incidence of RA is ~1% worldwide, but its etiology is not yet known. Several characteristics of RA, such as hyper-γ-globulinemia, autoantibody production, genetic linkage with the HLA-DR locus, and infiltration of T cells and plasma cells into the synovium, have suggested that immunological disorders play crucial roles in the pathogenesis of this disease (1). RA is a polygenic disease, and is caused by immunological disorders that develop from the synergistic actions of genetic and environmental factors, such as bacterial or viral infections. Clinical and experimental studies have revealed the involvement of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1, and IL-6 in the pathophysiology of RA (2). For example, TNF-α promotes the growth of synovial cells and blocking the TNF-α signal is an effective therapy (3).

IL-6 is a pleiotropic cytokine that regulates multiple biological functions, such as the development of the nervous and...
hematopoietic systems, acute-phase responses, inflammation, and immune responses (4). A causative role for IL-6 in autoimmune disease was first recognized because of the observation that the autoimmune symptoms of patients with cardiac myxoma, such as hyper-globulinemia and autoantibody production, disappeared with the surgical removal of the tumor which produced IL-6 (5). Furthermore, a high concentration of IL-6 was detected in the synovial fluid and serum of RA patients (6). Since then, clinical studies have provided more evidence supporting the involvement of IL-6 in the pathogenesis of autoimmune diseases (4,7). Studies using IL-6 knock-out mice revealed that IL-6 is involved in the severity and progress of experimentally induced arthritis (8–10). However, it was not known whether an abnormality in IL-6 family cytokine/gp130 receptor signaling was involved in spontaneous autoimmune diseases until we showed that a point mutation of the IL-6 family cytokine receptor, gp130, spontaneously caused RA-like autoimmune disease (11).

The IL-6 receptor consists of two molecules, the IL-6 receptor α chain and gp130, which is shared among the receptors for the IL-6 family cytokines. The ligand binding of gp130 activates two major signal-transduction pathways, the STAT3-mediated signal and the SHP-2/Gab/MAPK signal, in a manner dependent on the YXXQ motif and tyrosine (Y) 759 of gp130 respectively (12,13).

To clarify the roles played by the SHP-2- and STAT3-mediated signal-transduction pathways in vivo, we generated a series of knock-in mouse lines in which the gp130-mediated STAT3 or SHP-2 signals are selectively disrupted, by mutating the tyrosine residues of all the YXXQ motifs or Y759 to phospho-threonine (gp130FXXQ/FXXQ and gp130F759/F759 mice respectively). Our analysis of these mice indicated that the SHP-2-mediated or Y759-dependent signals negatively regulate the biological responses elicited by the STAT3-mediated signals in vivo, and that the balance of positive and negative signals generated through gp130 is skewed or shifted to positive STAT3 signaling in gp130FXXQ/FXXQ mice (14).

Importantly, the gp130F759/F759 mice spontaneously develop an RA-like autoimmune disease at ∼1 year of age (11). gp130F759/F759 mice show severe immunological abnormalities, including autoantibody production, increased memory/activated T cells, impaired thymic negative selection and peripheral clonal deletion. Most importantly, the development of the RA-like disease is totally dependent on mature lymphocytes.

HTLV-1 is the causative agent of adult T cell leukemia. HTLV-1 encodes a transcriptional trans-activator, Tax, in the env-pX region, that trans-activates transcription from the cognate viral promoter (15,16). Tax also activates many cellular genes for cytokines, cytokine receptors and immediately early transcriptional factors (17–20). HTLV-I is also associated with several chronic inflammatory diseases, such as HTLV-1-associated myelopathy/tropical spastic paraparesis and HTLV-I-associated arthropathy (21). The HTLV-1 transgenic mouse is an animal model of arthritis that is triggered by viral infection (22). This mouse develops a form of arthritis resembling human RA, accompanied by increased gene expression of inflammatory cytokines including IL-6, MHC molecules in the joints and resistance of T cells to Fas-mediated apoptosis (23). The susceptibility to arthritis is dependent on the genetic background of the mice; BALB/c mice are susceptible, but C57BL/6 mice are resistant to arthritis (24).

Here, we report clear synergy between the point mutation of Y759 of gp130 and the HTLV-1 pX gene in the C57BL/6 genetic background, in which the HTLV-1 pX gene alone cannot induce RA-like joint disease. The double-mutant mouse gp130F759/F759/pX-Tg developed severe arthritis much earlier than the gp130F759/F759 mouse. Lympho-hematopoietic abnormalities of the gp130F759/F759 mice, such as splenomegaly, lymphoadenopathy, hyper-globulinemia, autoantibody production, and increased granulocytes and memory/activated T cells, were augmented in the gp130F759/F759/pX-Tg mice. These abnormalities, as well as the onset, incidence and severity of the arthritis, were ameliorated in IL-6-deficient gp130F759/F759/pX-Tg mice. The gp130F759/F759/pX-Tg double-mutant mouse is a unique mouse model for the polygenic autoimmune disease RA.

Methods

Mice

gp130F759/F759 knock-in mice that had been backcrossed to C57BL/6 5 times or more were mated with HTLV-1-env-pX-transgenic mice (pX-Tg) that had been backcrossed 17 times to C57BL/6 (B6). The litters of the F1 generation were intercrossed to obtain gp130F759/F759/pX-Tg double mutants. Thus the double mutants were thought as backcrossed 6 times to B6 (N6). In all the analyses including the studies of clinical course, we used the wild-type and single-mutant mice derived from the same litters to generate the double- or triple-mutant mice.

IL-6-deficient mice were kindly given by Dr Kopf (25) and backcrossed 8 times to B6. They were then crossed with gp130F759/F759/pX-Tg+− to generate IL-6−/− gp130F759/F759/pX-Tg+− triple mutants, which were equivalent to 7 times backcrossed to B6 (N7). All these mice were kept at the Institute of Experimental Animal Sciences at Osaka University Medical School.

Analyses of arthritis

Clinical assessment of arthritis. Mice were inspected every week for up to 24 weeks and assessed for signs of arthritis: redness, swelling and restriction of mobility. The severity of the arthritis (arthritis score) was based on the restriction of mobility, and swelling of the wrist and ankle joints, which were each examined bilaterally. The severity of the arthritis was graded on a scale of 0–4 as follows: 0 (no change), 1 (minimal change), 2 (mild change), 3 (moderate change) and 4 (severe change). The arthritis score shown is a sum of the scores assessed bilaterally to give a possible maximum of 16 points for each mouse. The incidence was defined as the percentage of mice with a score ≥2 points.

Radiologic and histologic analyses of arthritis. Radiologic and histologic data from B6 gp130F759/F759/pX-Tg and 129/B6, gp130F759/F759 mice were obtained at 6–11 and 18 months of age respectively. X-ray photographs of the bones were taken using a Softex CMB-2 (Tokyo, Japan) and Fuji Film FR. For the histologic examination, joints were fixed in 10%
formalin/neutral phosphate buffer, decalcified in 10% EDTA-4Na and embedded in paraffin. Sections were stained with hematoxylin & eosin.

**Immunohistochemistry**

Synovium of the knee joint was excised and frozen in OCT compound using liquid nitrogen. Serial sections were cut at a thickness of 5 μm and fixed with acetone. After inactivation of the endogenous peroxidase with 0.01% H2O2 in PBS, the sections were blocked with 5% goat serum in PBS and then incubated with rat or hamster mAb, or rabbit antibody. Bound rat mAb was detected with horseradish peroxidase (HRP)-labeled goat anti-rat IgG (HistoOne; Nichirei, Japan) and visualized with 3,3′-diaminobenzidine (Dako, Kyoto, Japan).

Bound hamster mAb and rabbit antibodies were detected using a biotinylated mouse anti-hamster mAb mixture (PharMingen, San Diego, CA) and biotinylated goat anti-rabbit IgG (Zymed, South San Francisco, CA) respectively. For class II MHC staining, a biotinylated mouse anti-class II MHC mAb (25-9-17; PharMingen) was used. Bound biotinylated antibodies were visualized with ABC for HRP (Vector, Burlingame, CA) and 3,3′-diaminobenzidine or ABC for alkaline phosphatase (Vector) and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium. For nuclear counterstaining, hematoxylin or methyl green was used in single- or dual-staining experiments respectively. The antibodies used were: rat anti-mouse IL-6 (MP5-20F; PharMingen), rat anti-CD4 (RM4-5; PharMingen), anti-CD11b (M1/70) and anti-granulocytes (Gr-1, RB6-8C5), hamster anti-CD3 mAb (48-2B; Santa-Cruz, CA), and rabbit anti-phospho-STAT3 (Tyr705) (Cell Signaling, Beverly, MA). Anti-CD11b and Gr-1 were purified from the culture supernatant of hybridomas in our laboratory.

**Assay for serum antibodies and IL-6**

Serum IgG concentrations were measured with an ELISA system as described previously (26). For the concentrations of serum autoantibodies and IL-6, commercially available ELISA systems for rheumatoid factor of the IgG class (Shibayagi, Shibukawa, Japan), anti-dsDNA antibody (Shibayagi) and IL-6 (Biosource, Camarillo, CA) were used.

**Flow cytometry analysis**

A single-cell suspension prepared from the spleen or lymph nodes by teasing the tissue with slide glass was stained with a combination of labeled mAb, and analyzed with a FACS Calibur and CellQuest software, as described previously (27). The mAb used were: FITC-conjugated Gr-1 (a marker for granulocytes), IM7 (anti-CD44; PharMingen), PC61 (CD25; PharMingen), AM3 (IgM) and 25-9-17 (class II MHC, I-A^b; PharMingen), phycoerythrin (PE)-conjugated MEL14 (CD62L; Caltag, Burlingame, CA), H1.2F3 (CD69; PharMingen) and HL3 (CD11c; PharMingen), CyChrome-conjugated 53-6.7 (CD8; PharMingen) and RM4-5 (CD4; PharMingen), PE-Cy5-conjugated Gr-1 (Southern Biotechnology Associates, Birmingham, Alabama), TriColor-conjugated RA3-6B2 (CD45R; Caltag), allophycocyanin-conjugated M1/70 (CD11b; PharMingen), CTC4 (CD4; Caltag) and CTC8 (CD8; Caltag), biotinylated CS/70 (IgD), and CyChrome—
streptavidin (PharMingen). mAb Gr-1, AM/3 and CS/70 were purified from culture fluid supernatant and labeled with FITC or biotin by standard procedure.

Statistical analysis
Arthritis score, cell numbers of spleen and inguinal lymph nodes, ELISA assays, and frequencies of flow cytometry analyses of cell populations were compared by the Mann-Whitney U-test.

Results
Synergy between the Y759F mutation of gp130 and the HTLV-1 pX gene in the development of RA-like arthritis

pX-Tg mice in the C57BL/6 background (B6.pX-Tg mice) are resistant to arthritis (24). We generated double-mutant mice by crossing g130F759/F759 with B6.pX-Tg mice to determine the effects of pX gene expression with the g130F759/F759 mutation. The g130F759/F759/pX-Tg double mutants developed arthritis as young as 5 weeks old (Fig. 1). A 50% incidence of arthritis was observed at 11 weeks of age in B6.g130F759/F759/pX-Tg mice and within the 24-week observation period the incidence reached 70-100%. The incidences of male and female B6.g130F759/F759/pX-Tg mice at 24 weeks were 86 and 56% respectively. Only 7% of the B6.pX-Tg mice developed arthritis, with onset at ~16 weeks of age, consistent with the previous report that the C57BL/6 genetic background is resistant to the development of pX-dependent arthritis (24). Although the earliest symptom appeared in the B6.g130F759/F759 mice at ~10 weeks of age, 50% incidence was observed at ~38 weeks of age (data not shown), indicating that the progress of arthritis in the g130F759/F759/pX-Tg double mutants was much faster than in the single mutants. The average severity score of the g130F759/F759/pX-Tg double mutants gradually increased to 6 points (male 7 points and female 5 points), while that of the g130F759/F759 mice was <2 points within the 24-week observation period. These results indicate that the g130 Y759F mutation and pX gene synergize in the development and progress of arthritis. This synergistic effect was also observed in the double mutants generated by mating with g130F759/F759 mice backcrossed 8 times to B6, which generated the mutants with 9 times backcrossed to B6.

The earliest symptom of this arthritis was restriction of the movement of the wrist or ankle joints, which progressed to ankylosis with severe deformity of the wrists (not shown) and ankles (Fig. 2, top center). In radiologic analyses, the ankle joints of the B6.g130F759/F759/pX-Tg mice at 24 weeks of age (Fig 2, middle center) had erosions and deformity of bones, and ankylosis. Histological analysis of the g130F759/F759/pX-Tg mice at 24 weeks revealed hyperplasia of synovial fibroblasts, infiltration of neutrophils, narrowing of the joint spaces and ankylosis of the bone (Fig. 2, bottom center). These histological changes are similar to those observed in the arthritis of g130F759/F759 mice on the 129/B6 background at the age of 18 months (Fig. 2, right panels). Presence of the erosive arthritis and lack of nephropathy support this model to be RA-like rather than systemic lupus erythematosus-like, although both rheumatoid factor and anti-dsDNA antibody were detected in the sera.

To clarify the localization, interaction and activation status of the cells involved in this arthritis, we performed an immunohistochemical analysis of the synovium. Staining with
cells overlapped with a portion of the CD11b + cells (Fig. 3C). Cells were T cells (not shown). Very few CD8 + cells were observed in the superficial lining cells, while the independent synovial cells. Co-localization of CD11b and Gr-1 was layers of the synovial lining cells, indicating these were type A that cells expressing CD11b or Gr-1 were mainly located in the anti-CD11b (Fig. 3C) and anti-Gr-1 (Fig. 3B) mAb revealed Immunohistochemical analysis of the arthritic joints of sections (not shown), class II MHC+CD11b+ cells could be the gp130F759/F759 phosphorylated STAT3 (right; brown) in serial sections. producing cells (left; brown) and the nuclear localization of Y705 black arrow heads), (D) anti-IL-6 (brown; arrow heads) and anti-CD11b (brown; a white arrow head) and class II MHC (dark blue; stained with (A) hematoxylin & eosin, (B) anti-Gr-1 (brown), (C) anti-CD4 (brown: a white arrow head) and anti-class II MHC (dark blue; a black arrow head), and (F) IL-6-producing cells (left; brown) and the nuclear localization of Y705 phosphorylated STAT3 (right; brown) in serial sections.

anti-CD11b (Fig. 3C) and anti-Gr-1 (Fig. 3B) mAb revealed that cells expressing CD11b or Gr-1 were mainly located in the layers of the synovial lining cells, indicating these were type A synovial cells. Co-localization of CD11b and Gr-1 was observed in the superficial lining cells, while the independent localizations of CD11b+ or Gr-1+ cells were observed in the sublining area, representing macrophages and granulocytes. There were areas containing scattered CD4+ cells that overlapped with regions dominated by CD11b+ cells in the sublining (Fig. 3C and E). Dual staining showed co-localization of CD3+ cells with CD4+ cells, confirming that most of the CD4+ cells were T cells (not shown). Very few CD8+ cells were observed (not shown). The area containing CD4+ cells was closely located around or overlapping the area containing class II MHC+ cells (Fig. 3E), suggesting this to be a location where certain immune responses take place. Class II MHC+ cells overlapped with a portion of the CD11b+ cells (Fig. 3C). Since CD11c+ cells were rarely observed in the synovial sections (not shown), class II MHC+CD11b+ cells could be the main cell population presenting antigens in the synovium. We then examined the production of inflammatory cytokines. An ELISA of serum IL-6 levels in gp130F759F759/pX-Tg mice aged 4–5 months old (101 ± 38 pg/ml; average ± SEM of n = 9) showed a 31 or 53% increase compared with pX-Tg (78 ± 32 pg/ml; n = 7) or gp130F759F759 (66 ± 36 pg/ml; n = 7) mice respectively. Although these differences are not significant, it suggests that local IL-6 production is augmented in gp130F759F759/pX-Tg mice, probably due to the action of Tax, as reported previously (18). Immunohistochemically, local IL-6 production was observed at the superficial lining layers extending to the sublining areas in the arthritic synovium (Fig. 3D and F, left). The stronger staining at the sublining area and the shape of the cells indicate that synovial fibroblasts were the major IL-6 producers (Fig. 3F, left). IL-1β (not shown) and TNF-α (Fig. 3D) were faintly detected, but the staining was much weaker than that of IL-6, suggesting that IL-6 is the major inflammatory cytokine in this arthritis. To detect IL-6-mediated signaling, we examined the localization of phospho-STAT3. Some cells that were located in the area of IL-6 production also showed nuclear localization of phospho-STAT3 (Fig. 3F, right), suggesting that these cells were receiving aberrant gp130 signals from IL-6 produced at the site.

Synergy between the gp130 Y759F mutation and HTLV-1 pX for the generation of arthritis was dependent on IL-6. Among the IL-6 family cytokine members that use gp130 as a signal-transducing receptor subunit, IL-6 plays a major role in the regulation of immune responses (28). Furthermore, the immunohistochemical analyses in the present study demonstrated that IL-6 was produced at the synovium in the arthritic joints and activated STAT3-mediated signaling. Thus, we examined the roles of IL-6 in the pathogenesis of the severe arthritis in gp130F759F759/pX-Tg double mutants by generating IL-6±/±/gp130F759F759/pX-Tg triple mutants.

As shown in Fig. 4(A), arthritis in the gp130F759F759/pX-Tg double mutants started to appear in mice at 7 weeks old, whereas that of the IL-6+/−gp130F759F759/pX-Tg triple mutants started in mice at 16 weeks old, 9 weeks later. The incidence of arthritis in the IL-6+/−/gp130F759F759/pX-Tg triple mutants then gradually increased, but at 24 weeks, when the incidence of arthritis in the gp130F759F759/pX-Tg double mutants had reached 100%, that in the IL-6+/−/gp130F759F759/pX-Tg triple mutants showed a plateau at 50%. Furthermore, the average severity score of the IL-6+/−/gp130F759F759/pX-Tg triple mutants was much lower than that of the gp130F759F759/pX-Tg double mutants (2 versus 6). These data indicated that IL-6 plays pivotal roles in determining the time of onset, final incidence and severity of the arthritis in the double mutants. Histological examination revealed that hyperplasia of the synovial fibroblasts, infiltration of neutrophils and bone destruction were much ameliorated in the IL-6+/−/gp130F759F759/pX-Tg triple mutants (Fig. 4B, top). Radiologic analysis showed that the osteoporotic changes, erosive changes of the toes and reactive calcification of the tarsal bones seen in the gp130F759F759/pX-Tg mice were not observed in the IL-6+/−/gp130F759F759/pX-Tg triple mutants (Fig. 4B, bottom).

Immunological abnormalities caused by the synergy between the gp130Y759F mutation and HTLV-1 pX were dependent on IL-6. To clarify the role of IL-6 in the arthritis of gp130F759F759/pX-Tg double-mutant mice, various parameters of the lympho-
hematopoietic systems of each mouse were examined and compared. The average age of the mice in the groups analyzed was 32 weeks (range 24–40 weeks) and the genotypes and numbers of mice used were: gp130\(^{WT/WT}\)/pX-Tg\(^{-/-}\) (n = 6), gp130\(^{F759/F759}\)/pX-Tg\(^{-/-}\) (n = 5), gp130\(^{WT/WT}\)/pX-Tg\(^{+/+}\) (n = 5), gp130\(^{F759/F759}\)/pX-Tg\(^{+/+}\) (n = 5), and IL-6\(^{-/-}\)/gp130\(^{F759/F759}\)/pX-Tg\(^{+/+}\) (n = 5). The average severity score of gp130\(^{F759/F759}\)/pX-Tg (11.6 ± 1.2) was much higher than that of the arthritic mice gp130\(^{F759/F759}\)/pX-Tg (1.4 ± 0.9) and that of IL-6\(^{-/-}\)/gp130\(^{F759/F759}\)/pX-Tg was significantly decreased (4.0 ± 1.6) (Fig. 5A).

Similar to the severity score, splenic cell number (Fig. 5B) and the titer of rheumatoid factor (Fig. 5E) of gp130\(^{F759/F759}\)/pX-Tg were significantly higher than those of wild-type or single mutants. The cell numbers of inguinal lymph node (Fig. 5C) and the concentrations of serum IgG (Fig. 5D) and anti-dsDNA antibody (Fig. 5F) showed increased values in the gp130\(^{F759/F759}\)/pX-Tg mouse group both in the presence or absence of pX.
expression, indicating these changes are dependent on the gp130F759/F759 mutation. These parameters, except for the inguinal lymph node cell numbers, normalized in the absence of IL-6, indicating significant IL-6 dependency.

Then we performed flow cytometry analyses to investigate the IL-6-dependent changes in the immune-competent cell populations of the inguinal lymph nodes (Fig. 6 and Table 1). Around 7 months old, only the reduction of naive CD4 T cells (CD62L+CD44−) and slight increases of activated CD4 T cells (CD62L+CD44+ or CD69+) were observed in gp130F759/F759 mice (Fig. 6A). In contrast, marked decreases of naive CD4 and CD8 T cells, and increases of memory/activated CD4+ T cells (CD62L+CD44+, CD62L−CD44+ and CD69+ cells), activated CD8+ T cells (CD62L−CD44+ and CD69+ cells) and granulocytes (CD11b+Gr-1+) were observed in gp130F759/F759/pX-Tg, whereas these changes were almost normal in IL-6−/− gp130F759/F759/pX-Tg−/− (Fig. 6A and B, and Table 1), suggesting that these changes are IL-6 dependent, and intimately related to autoimmunity and the severity of arthritis.

Since T cell activation is mainly regulated by professional antigen-presenting cells (29), we examined the dendritic cells in the lymph nodes of arthritic mice (Fig. 6B and Table 1). The average frequencies of CD11c+ cells in the lymph nodes of wild-type and gp130F759/F759/pX-Tg mice were 5 and 6%

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**Fig. 5.** IL-6 dependency of various parameters examined in the analysis of gp130F759/F759/pX-Tg−/−. The arthritis score (A), total cell number in the spleen (B) and bilateral inguinal lymph nodes (C), and concentrations of serum IgG (D), rheumatoid factor (E) and anti-dsDNA antibody (F) in mice averaging 32 weeks old (range 24–36) of the following genotypes are shown. The numbers on the horizontal axis indicate: 1, gp130WT/WT/pX-Tg−/− (n = 6); 2, gp130F759/F759/pX-Tg−/− (n = 6); 3, gp130F759/F759/pX-Tg+/− (n = 5); 4, gp130F759/F759/pX-Tg+/+ (n = 8); and 5, IL-6−/− gp130F759/F759/pX-Tg−/− (n = 5). The circles and horizontal bars indicate the values for each mouse and the average respectively. *P < 0.05; **P < 0.01
respectively, indicating that this double mutation did not greatly affect the development of dendritic cells. However, the expression levels of class II MHC (CIIMHC) molecules were drastically changed in the gp130F759/F759/pX-Tg dendritic cells. Among CD11c+ cells, mature dendritic cells can be distinguished by the bright expression of class II MHC. In wild-type control mice, the CIIMHCbright population represented 35% of the CD11c+ cells, whereas in gp130F759/F759/pX-Tg mice it was only 4%. Furthermore, of the IL-6±/±gp130F759/F759/pX-Tg CD11c+ cells, the CIIMHCbright population recovered to 29%, indicating that the decreased frequency of the CIIMHCbright population was dependent on IL-6/gp130 signaling. A similar reduction of the CIIMHCbright population was also observed in the CD11c+ cells of gp130F759/F759/pX-Tg mice at 16 weeks of age, before the increase of memory or activated T cells was beginning to be obvious. Since this change of T cells follows a decreased frequency of CIIMHCbrightCD11c+ cells among lymph node cells, it may affect the regulation of T cell responses.

Discussion
We recently reported that a point mutation of gp130, a signal-transducing receptor subunit common to IL-6 family cytokines, causes RA-like autoimmune arthritis. This model, the gp130F759/F759 mouse, is unique and important because it provided the first experimental evidence that a point mutation of a cytokine receptor could cause autoimmune disease. Furthermore, the autoimmune disease spontaneously developed as a result of deregulated IL-6/gp130 signaling. In the gp130F759/F759 mouse, a point mutation of gp130 Y759F selectively disrupts the SHP-2-mediated gp130 signals, which results in the loss of ERK activation and in the prolonged activation of STAT3. Such unbalanced signaling is likely to be involved in triggering the breakdown of self-tolerance.

In this paper, we showed a clear synergy between the gp130 Y759F mutation and the HTLV-1 pX gene in causing RA-like arthritis, i.e. a genetic factor (the point mutation of gp130) and an environmental factor (the overexpression of pX, which mimics HTLV-1 infection) synergize, and facilitate the onset and progress of arthritis. It was surprising that the combination of a genetic factor for arthritis with late onset and slow progression, and a virus gene expression that evokes arthritis only rarely in a B6 genetic background, resulted in arthritis that had much earlier onset and faster progress than was seen in either single mutant. In the gp130F759/F759/pX-Tg mouse, abnormalities previously observed in gp130F759/F759 mice were augmented. These include splenomegaly,
lymphoadenopathy, hyper-\(\gamma\)-globulinemia, autoantibody production, increases in granulocytes and memory/activated T cells, and decreases in naive T cells and class II MHC\textsuperscript{bright}CD11c\textsuperscript{+} cells. The clinical course of the triple mutant, IL-6\textsuperscript{--/--} gp130\textsuperscript{F759/F759}pX-Tg, was markedly ameliorated, indicating that the acceleration of arthritis in gp130\textsuperscript{F759/F759}pX-Tg is largely dependent on IL-6. Furthermore, almost all of the abnormalities seen in the gp130\textsuperscript{F759/F759}pX-Tg mice were found to be dependent on IL-6, suggesting that these abnormalities are intimately related to the onset or progress of the disease.

Although we did not clarify the molecular mechanisms for the acceleration of the arthritis, we can speculate that the mutations in gp130 and the pX gene play distinct roles in this process. Since the latest analysis of arthritis in 8 times backcrossed B6.gp130\textsuperscript{F759/F759} mice revealed that the incidence reached 100\% at 54 weeks (unpublished observation), the major effect of the pX gene seems to be to accelerate the progression of the disease. The increased cytokine gene expression in pX-Tg reported previously supports this notion.

In particular, IL-6 gene expression is known to be enhanced by pX and the synergy we observed was dependent on IL-6; therefore, it is likely that a pX-induced up-regulation of the IL-6 gene is one of the mechanisms. Since the major effect of pX expression is mediated by NF-\(\kappa\)B activation, a role for pX could be as a substitute for TNF-\(\alpha\) or IL-1 in virus-mediated effects.

On the other hand, because B6.pX-Tg rarely develops arthritis, the role of Y759F-mutated gp130 seems to be to trigger the disease in the pX-Tg mutant of an arthritis-resistant strain. Although IL-6 gene targeting in gp130\textsuperscript{F759/F759}pX-Tg could not completely block the development of arthritis, the incidence reached a plateau at 50\%, suggesting the involvement of other IL-6 family cytokines that utilize gp130.

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**Abbreviations**

- CII: class II
- HRP: horseradish peroxidase
- PE: phycoerythrin
- RA: rheumatoid arthritis
- Tg: transgenic
- TNF: tumor necrosis factor

**Fig. 6.** IL-6-dependent changes of immune-competent cell populations in the inguinal lymph nodes of gp130\textsuperscript{F759/F759}pX-Tg\textsuperscript{--}. The inguinal lymph node cells of each genotype at 7 months old were stained with the combination of mAb and analyzed with a flow cytometer as described in Methods. (A) Analyses of T cell subsets. (B) Analyses of myeloid cells. Numbers in the quadrants of dot-plots for CD4/CD8 and Gr-1/CD11b indicate the frequencies in total inguinal lymph node cells. Numbers in the quadrants of dot-plots for CD44/CD62L and CD25/CD69 indicate the frequencies in CD4\textsuperscript{+} or CD8\textsuperscript{+} inguinal lymph node cells. Numbers in the regions of dot-plots for CD11c/CIIMHC indicate the frequency of CD11c\textsuperscript{+} cells in the inguinal lymph node cells. In the histogram, the frequencies of CIIMHC\textsuperscript{bright} cells in the CD11c\textsuperscript{+} cells are indicated. Representative data from the analyses summarized in Table 1 are shown.
Table 1. Flow cytometry analysis of the lymph node cells of gp130<sup>F759F759</sup>pX-Tg<sup>±±</sup>.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>gp130/pX/IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W/W/W</td>
</tr>
<tr>
<td>CD4</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>CD62L&lt;sup&gt;d&lt;/sup&gt;-CD44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>CD62L&lt;sup&gt;d&lt;/sup&gt;-CD44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>CD69&lt;sup&gt;d&lt;/sup&gt;-CD25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>CD8</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>CD62L&lt;sup&gt;d&lt;/sup&gt;-CD44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>CD62L&lt;sup&gt;d&lt;/sup&gt;-CD44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>CD69&lt;sup&gt;d&lt;/sup&gt;-CD25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>CD45R</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;d&lt;/sup&gt;-Gr-1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;d&lt;/sup&gt;-Gr-1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>CD11c&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>C1LIMH&lt;sub&gt;bright&lt;/sub&gt;</td>
<td>35 ± 2</td>
</tr>
</tbody>
</table>

<sup>a</sup> The inguinal lymph node cells of each genotype at 7 months old, as described in the Results and Fig. 5, were stained with the combination of mAb and analyzed with a flow cytometer as described in Methods. The abbreviations for the genotypes are: W/W/W, F/W/W, F/Tg/W, F/Tg/K for gp130<sup>F759F759</sup>pX-Tg<sup>±±</sup>, gp130<sup>F759F759</sup>pX-Tg<sup>±±</sup> and IL-6<sup>b</sup>/gp130<sup>F759F759</sup>pX-Tg<sup>±±</sup> respectively. Numbers indicate average frequencies ± SEM of each population in the inguinal lymph node cells except for those described in 'b'.

<sup>b</sup> The frequencies in the CD4<sup>c</sup> or CD8<sup>d</sup> inguinal lymph node cells are used for the cell populations defined by CD44/CD62L or CD25/CD69 expression, whereas the frequencies in the CD11c<sup>d</sup> cells are used for C1LIMH<sub>bright</sub>CD11c<sup>d</sup> cells.

<sup>c</sup> Pairs of bold numbers in each population indicate that they are significantly different (p < 0.05).

<sup>d</sup> The frequencies returned to nearly normal in the absence of IL-6.

NE: not examined.

References