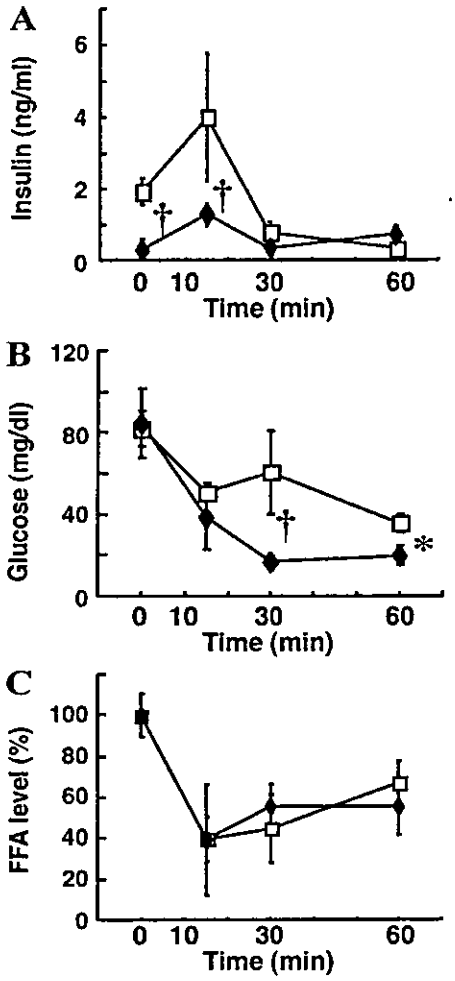

Figure 6. Reduction of insulin levels in IL-1Ra$^{-/-}$ mice. Insulin levels after glucose tolerance test (A), and glucose (B) and FFA (C) levels after insulin tolerance test in wild-type (open squares) and IL-1Ra$^{-/-}$ (closed diamonds) mice. Relative FFA levels are expressed as the percentage relative to the value at time 0 (before insulin administration). Data are expressed as the average ± SD. The results are reproducible in three independent experiments using at least four mice for each genotype. Statistical significance was determined by Student's *t* tests. *, $P < 0.05$, †, $P < 0.01$ versus wild-type mice.

Effect of Limited Diet on Body Weight Maintenance. We analyzed the energy storage efficiency of these mutant mice by measuring body weight changes in response to food restriction. When daily food was restricted to 0.9 g of a normal chow, the body weight of wild-type mice decreased to two-thirds of the initial weight after 14 d and maintained that level until 18 d (Fig. 3). Under ad libitum feeding conditions, wild-type mice eat 3.2 ± 0.4 g/day, whereas mutant mice eat 2.8 ± 0.2 g/day. Interestingly, similar body weight loss was also observed in food-restricted IL-1Ra$^{-/-}$ mice, and the body weight of IL-1Ra$^{-/-}$ mice still remained significantly lower than that of wild-type mice. These results clearly demonstrate that IL-1Ra$^{-/-}$ mice have defects in lipid storage even when they consume the same amount of food as wild-type mice, which is inconsistent with the notion that the lean phenotype of this mutant mouse is not caused by a central mechanism but by peripheral mechanisms.

Lipid Utilization in IL-1Ra$^{-/-}$ Mice. Next, we studied mice that were fed a high-fat, normocaloric diet, or a normal chow for 8 wk. Body weight did not differ between the high-fat diet group and the normal chow group, suggesting that mice from both groups obtained equivalent amounts of energy from these diets (Fig. 4 A). However, epididymal WAT mass was significantly increased in wild-type mice fed the high-fat diet (Fig. 4 B). In contrast, IL-1Ra$^{-/-}$ mice fed the high-fat diet did not gain additional adipose mass (Fig. 4 B). Serum TAG levels in these mice reached the same levels as in wild-type mice, although the levels in mice fed normal diet were significantly low (Fig. 4 C). These results suggest that lipid uptake into adipose tissues is impaired in IL-1Ra$^{-/-}$ mice.

Blood Constituents in IL-1Ra$^{-/-}$ Mice under Physiological Conditions and after Refeeding. To examine possible involvement of the endocrine system in the deficiency of lipid uptake seen in IL-1Ra$^{-/-}$ mice, we measured basal levels of glucose, insulin, leptin, TAG, TC, and FFA. Serum insulin, leptin, and TAG levels in IL-1Ra$^{-/-}$ male mice were significantly lower than those of wild-type mice under fed conditions (Fig. 5). On the other hand, levels of other blood constituents, including glucose, TC, and FFA, were comparable between wild-type and IL-1Ra$^{-/-}$ male and female mice (TC wild-type mice: male [92 ± 19 mg/dl], $n = 5$ [female, 68 ± 13 mg/dl, $n = 4$]; IL-1Ra$^{-/-}$ mice: male [85 ± 11 mg/dl, $n = 5$], female [72 ± 19 mg/dl, $n = 6$]; FFA wild-type mice: male [1.04 ± 0.12 mEq/l, $n = 5$], female [0.91 ± 0.03 mEq/l, $n = 4$]; IL-1Ra$^{-/-}$ mice: male [0.91 ± 0.14 mEq/l, $n = 5$], female [1.18 ± 0.04 mEq/l, $n = 6$]; average ± SD).

After a 48-h fast, blood constituents were periodically measured after refeeding, using weight-matched wild-type and IL-1Ra$^{-/-}$ mice, to avoid a possible confounding influence of body weight on recovery. In this experiment, body weight and food intake were measured under fed, fasted, and refed conditions. IL-1Ra$^{-/-}$ mice consumed as much food as wild-type mice during refeeding, reconfirming that appetite was not reduced in these mice (unpublished data). Although glucose levels were normal, insulin, TAG, and leptin levels in response to refeeding were altered in IL-1Ra$^{-/-}$ mice (Fig. 5). Decreased levels of serum TAG and leptin in IL-1Ra$^{-/-}$ mice persisted for 4 h after refeeding, even though mutant mice consumed the same amount of food as wild-type mice (Fig. 5, C and D).

Interestingly, insulin levels were significantly low in IL-1Ra$^{-/-}$ male mice under physiological conditions. This trend was more clearly apparent under fasted conditions. Upon refeeding, insulin levels in IL-1Ra$^{-/-}$ mice were also significantly lower than in control mice, with a delayed peak in insulin secretion after refeeding (Fig. 5 B). Similar results were obtained when age-matched mice were used

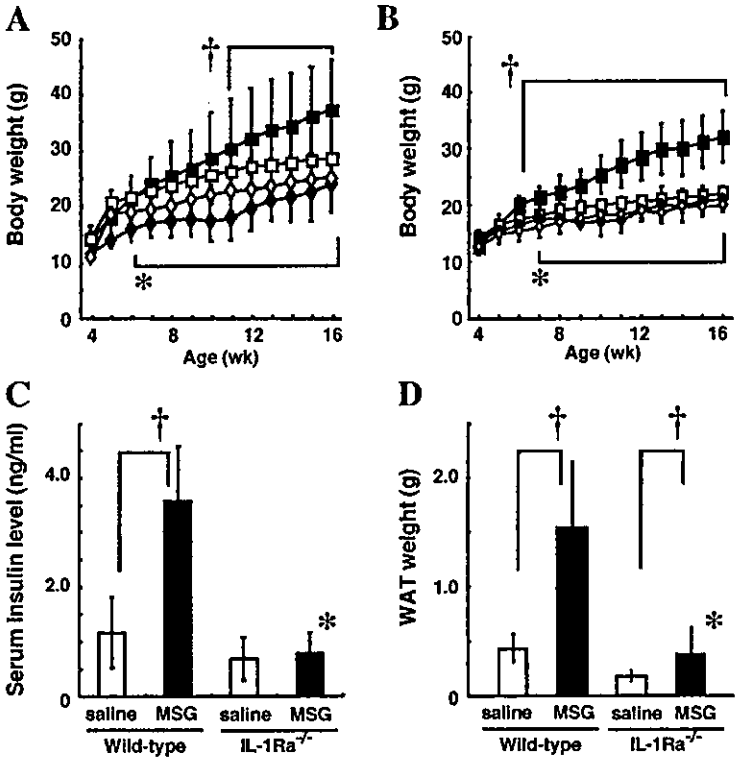

Figure 7. Resistance of IL-1Ra$^{-/-}$ mice to MSG-induced obesity. Total body weights of male (A) and female (B) control (wild-type and IL-1Ra$^{+/-}$) mice (squares) and IL-1Ra$^{-/-}$ mice (diamonds) after MSG (closed symbols) or saline (open symbols) treatment were measured (MSG-treated control mice: male, $n = 6$, female, $n = 8$; saline-treated control mice: male, $n = 13$, female, $n = 10$; MSG-treated IL-1Ra$^{-/-}$ mice: male, $n = 9$, female, $n = 5$; saline-treated IL-1Ra$^{-/-}$ mice: male, $n = 13$, female, $n = 5$). Serum insulin levels (C) and WAT weight from epididymis (D) of male control mice and IL-1Ra$^{-/-}$ mice at 20 wk old after MSG (shaded bars) or saline (white bars) treatment. Data are expressed as the average ± SD. Statistical significance was calculated by repeated measures ANOVAs and Tukey post hoc tests. *, $P < 0.05$ MSG-treated IL-1Ra$^{-/-}$ mice versus MSG-treated wild-type mice. †, $P < 0.05$ MSG-treated versus saline-treated mice.

for the experiments (unpublished data). These observations indicate that IL-1Ra deficiency affects insulin levels in the circulation. Although we do not know whether this is due to suppression of the insulin production or secretion, or even acceleration of the clearance at this moment, we use the phrase "insulin secretion" as it represents the sum of these processes in the following sections.

The effects of IL-1Ra deficiency on insulin secretion and insulin sensitivity were examined by glucose tolerance test and insulin tolerance test, respectively. After administration of glucose, the insulin response was significantly reduced in IL-1Ra$^{-/-}$ mice relative to wild-type mice (Fig. 6 A), although blood glucose levels were not significantly different between IL-1Ra$^{-/-}$ and wild-type mice (unpublished data). Furthermore, the sensitivity of blood glucose levels to insulin was significantly increased in IL-1Ra$^{-/-}$ mice (Fig. 6 B), although the effect of insulin on FFA release was similar between IL-1Ra$^{-/-}$ and wild-type mice (Fig. 6 C). The defect in insulin secretion in mutant mice was not due to developmental defects, because the β cells appeared normal by hematoxylin/eosin and β cell-specific staining (unpublished data). These results indicate that IL-1Ra deficiency affects lipid metabolism and insulin secretion independently from feeding behavior.

MSG-induced Obesity and Metabolic Disorders in IL-1Ra$^{-/-}$ Mice. MSG treatment ablates cells in the arcuate nucleus in the hypothalamus (ARH), which are involved in the metabolic regulation and leptin signaling (40), and this treatment induces maturity-onset obesity in wild-type mice without affecting appetite (36, 39, 41). Because IL-1RI mRNA is expressed in the ARH (9, 42), we examined the possibility that the action of IL-1 may be mediated by the neurons in the ARH. As shown in Fig. 7 (A and B), both male and female IL-1Ra$^{-/-}$ mice showed complete resistance to obesity in contrast to wild-type mice. WAT weight was increased, although much less than MSG-treated wild-type mice (Fig. 7 C). Interestingly, serum insulin level was not elevated in IL-1Ra$^{-/-}$ mice in clear contrast to wild-type mice (Fig. 7 D). These results indicate that excess IL-1 signaling generated in IL-1Ra$^{-/-}$ mice antagonizes the effect of ARH lesion.

Effect of IL-1Ra Deficiency on Lipase Activity. To further demonstrate the effects of IL-1Ra deficiency on lipid metabolism, we studied lipase activity in IL-1Ra$^{-/-}$ mice. In the plasma of IL-1Ra$^{-/-}$ mice after heparin administration, total lipase activity, but not hepatic lipase activity, was significantly reduced compared with wild-type mice (Fig. 8). These observations suggest that IL-1 is also involved in the regulation of lipase activity under physiological conditions.

Discussion

In this paper, we examined the physiological role of IL-1 on feeding behavior and energy metabolism using IL-1$^{-/-}$ and IL-1Ra$^{-/-}$ mice. We found that IL-1Ra$^{-/-}$ mice have a defect in lipid accumulation in adipose tissue, although IL-1$^{-/-}$ mice do not show any apparent abnormalities. Thus, IL-1 signal is not necessarily required for the lipid

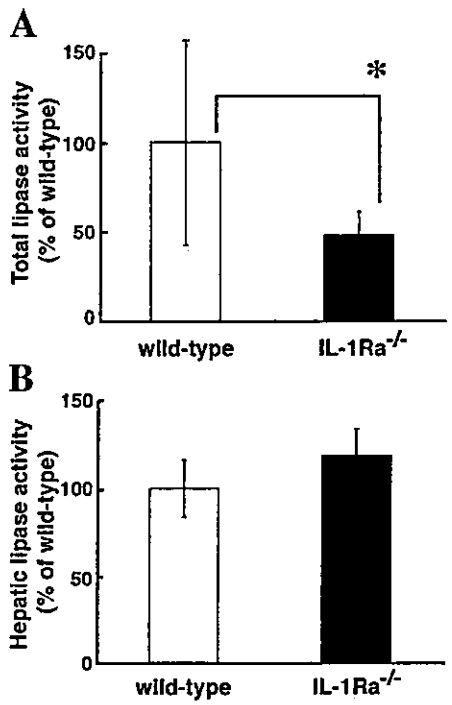

Figure 8. Reduced PHP lipase activity in IL-1Ra$^{-/-}$ mice. Mice were administered 100 U/kg heparin intravenously, and plasma were collected after 5 min (A) total lipase activity and (B) hepatic lipase activity of the PHP from age-matched wild-type (white bars, $n = 8$) and IL-1Ra$^{-/-}$ (shaded bars, $n = 5$) male mice. Data are expressed as the average ± SD. Statistical significance was determined by Student's t tests. *, $P < 0.05$ versus wild-type mice.

metabolism, but its excess signaling is harmful for the energy homeostasis of the body. The defect was more severe in males than in females, probably reflecting hormonal differences.

It is well-known that IL-1 is involved in fever, anorexia (loss of appetite), and cachexia that develop during infection, inflammation, cancer, or physical stress (4, 43). Fever and feeding suppression caused by leptin are also mediated by hypothalamic IL-1 (44). IL-1 can activate POMC neurons in the ARH where IL-1RI mRNA is expressed (9, 42), and the anorexic, but not pyrogenic, actions of IL-1 are mediated by central MC3/4Rs (23). Recently, two groups reported that cachexia was ameliorated by central MC3/4R blockade, indicating cancer anorexia is also mediated by central melanocortin pathway (45, 46). Furthermore, it was reported that leptin was induced by LPS through induction of IL-1 (47). Thus, it seemed likely that leanness of IL-1Ra$^{-/-}$ mice might be resulted from feeding suppression mediated by the ARH–melanocortin pathway. However, our findings suggest that the lean phenotype of IL-1Ra$^{-/-}$ mice is not caused by feeding suppression. This is because food intake per gram body weight is normal, and mutant mice showed a lean phenotype even when they were fed the same amount as wild-type mice. IL-1Ra$^{-/-}$ mice show normal energy expenditure and heat production. Furthermore, expression levels of major hypothalamic factors involved in the melanocortin system are normal. Thus, we conclude that leanness of IL-1Ra$^{-/-}$ mice results not from feeding suppression but from metabolic disorder in the periphery that is caused directly by excess IL-1 signaling or indirectly through central mechanisms. IL-1 signaling under physiological conditions may be too weak to evoke feeding suppression, and only a large excess IL-1 signaling, such as that produced under pathological conditions, may suppress appetite through a hypothalamic mechanism.

We have shown that IL-1$^{-/-}$ mice do not show any apparent abnormality in feeding behavior or body temperature under physiological conditions. Consistent with our observations, it was reported that mice doubly deficient for the CRF RI and RII, which function down-stream of IL-1 in the hypothalamus, do not show any abnormalities under physiological conditions (22). Furthermore, IL-1Ra transgenic mice, with either the endogenous IL-1Ra promoter or the glial fibrillary acidic protein promoter, did not show any alterations in body weight (48, 49). Chronic central administration of IL-1Ra also did not affect food intake and weight gain in rats (50). Collectively, these observations indicate that IL-1 is not necessarily required for the control of appetite or body temperature under physiological conditions, although it plays most important roles under pathological conditions.

IL-1 reportedly suppresses intestinal lipid absorption and lipid accumulation in vivo, although the mechanism has not been completely elucidated (51). Although serum TAG levels are low in IL-1Ra$^{-/-}$ mice, this is not a result of defects in intestinal lipid absorption, because TAG levels in the chylomicron fraction of serum lipoproteins in IL-1Ra$^{-/-}$ mice are similar to those in wild-type mice (unpublished data). On the other hand, lipid accumulation is inhibited in mutant mice, because these mice show decreased fat accumulation in adipose tissue even when fed a high-fat diet, which leads to serum TAG levels similar to wild-type mice. The ability of embryonic fibroblasts to differentiate into mature adipocytes in vitro, however, is normal in IL-1Ra$^{-/-}$ mice, indicating that the ability of adipocyte progenitor cells to differentiate into mature adipocytes is normal in mutant mice (unpublished data). Furthermore, fatty acid uptake by in vitro–differentiated adipocytes from IL-1Ra$^{-/-}$ mice is also normal. Therefore, these observations suggest that IL-1 does not directly affect the differentiation or function of adipocytes, but rather affects adipocyte function by an indirect mechanism.

In this context, it is noteworthy that serum insulin levels in IL-1Ra$^{-/-}$ mice are significantly low under free-fed conditions and during recovery from starvation. Impaired insulin secretion is also observed after glucose administration. Furthermore, insulin secretion upon glucose administration is suppressed by the administration of IL-1 in wild-type mice (unpublished data), in agreement with previous works (52, 53). These findings indicate that excess IL-1 signaling suppresses insulin secretion from the pancreas.

Insulin is a major regulator of lipid metabolism in adipocytes, and it promotes adipocyte TAG store by fostering

the differentiation of preadipocytes, stimulating glucose transport and TAG synthesis, and inhibiting lipolysis (54). Insulin also increases the uptake of fatty acids derived from circulating lipoproteins by stimulating LPL activity (55–57) and promoting the trafficking of fatty acid transporters in adipose tissue (58). Actually, we showed that PHP lipase activity is reduced in IL-1Ra$^{-/-}$ mice. Thus, decreased insulin may cause reduced fat accumulation in adipose tissue of IL-1Ra$^{-/-}$ mice. It is also possible that excess IL-1 signaling affects LPL activity resulting in the suppression of fat accumulation.

However, normal serum glucose levels are maintained in IL-1Ra$^{-/-}$ mice under physiological conditions, despite decreased insulin levels. This is because insulin sensitivity is increased in IL-1Ra$^{-/-}$ mice as monitored by insulin tolerance tests. In contrast, the sensitivity of serum FFA to insulin is not increased in IL-1Ra$^{-/-}$ mice, and the expression of adiponectin and resistin, which are involved in the insulin sensitivity of adipose tissue (59–61), are not changed. These results indicate that the sensitivities of serum glucose and FFAs to insulin are different, and only lipid metabolism may be affected by the deficiency of insulin levels in IL-1Ra$^{-/-}$ mice.

It is known that MSG-sensitive neurons are involved in the peripheral lipid metabolism because disruption of these neurons causes obesity in wild-type mice, probably by activating the vagus nerves without affecting food intake (62). It is also known that MSG treatment activates insulin secretion in wild-type mice (63). In contrast, disruption of the ARH neurons by MSG treatment did not cause obesity in IL-1Ra$^{-/-}$ mice. Serum insulin levels were also not increased in these MSG-treated mutant mice, indicating that excess IL-1 signaling antagonizes the effects of the ARH neuron damage. However, it remains to be elucidated whether IL-1 acts on the pancreas so as to antagonize the effect of vagus nerve activation or directly suppresses vagus nerve activation. Nonetheless, these observations support the notion that IL-1 suppresses lipid accumulation in peripheral tissues by reducing blood insulin levels.

We reported previously that IL-1Ra$^{-/-}$ mice on a BALB/cA background spontaneously develop chronic inflammatory arthropathy resembling rheumatoid arthritis, after weaning (64). On the C57BL/6J background, these mutant mice, however, scarcely develop arthritis even at an older age (>24 wk old; reference 64). Because no IL-1Ra$^{-/-}$ mice on this genetic background develop arthritis at 5 wk old, whereas the lean phenotype develops as early as 5 wk old, leanness is not likely to be caused by autoimmunity or inflammation.

In summary, we have shown that the lean phenotype of IL-1Ra$^{-/-}$ mice is not caused by feeding suppression, but rather by impaired lipid accumulation. We showed that IL-1Ra$^{-/-}$ mice exhibit defects in postprandial insulin secretion and lipid metabolism. These results indicate that the IL-1 system plays a pivotal role in maintaining insulin homeostasis under physiological conditions. The IL-1Ra$^{-/-}$ mouse is a unique model for leanness and should be of use to further investigate obesity, diabetes, and lipid metabolism disorders.

We thank Dr. Tsubone for supporting the experiments of energy expenditure. We also thank the members of our laboratory for helpful discussions and assistance in animal care.

This work was supported by grants from the Ministry of Education, Science, Sport and Culture of Japan, the Ministry of Health and Welfare of Japan and the Pioneering Research Project in Biotechnology.

Submitted: 24 February 2003
Revised: 5 June 2003
Accepted: 2 July 2003

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Gann Monograph on Cancer Research 50, 2003

AUTOIMMUNE CHRONIC INFLAMMATORY ARTHROPATHY IN MICE TRANSGENIC FOR THE HTLV-I *tax* GENE

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Rheumatoid arthritis (RA) is a serious medical problem, with ~1% of the people in the world affected. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissue in multiple joints, which leads to joint destruction. It is remarkable that expression of inflammatory cytokines is augmented in the joints of the patients, although the pathological roles have not been elucidated completely. We recently reported on an inflammatory arthropathy resembling RA that develops in high incidence among transgenic (Tg) mice that carry the *env-pX* region of the human T cell leukemia virus type I (HTLV-I) genome. Autoimmune pathogenesis was suggested in this RA model because: 1) high levels of autoantibodies were detected in the serum, 2) oligoclonal accumulation of T cells was detected in the affected joints, 3) the development of arthritis was suppressed in athymic nude mice, and 4) the disease was transferred by bone-marrow (BM) cell transplantation and suppressed by wild-type BM cell transplantation. We found that cytokine levels including interleukin (IL)-1 were elevated in the joints of these Tg mice. Depletion of IL-1 by gene targeting greatly reduced onset of the disease and T cell proliferative response against synovial components was also reduced, indicating importance of this cytokine in the development of arthritis and autoimmunity. Furthermore, we found that IL-1 receptor antagonist (IL-1Ra)-deficient mice also developed arthritis spontaneously, and autoimmune nature of the disease was suggested. These observations suggest that excess IL-1 signal causes autoimmunity. We show that IL-1 induces expression on T cells of CD40L and OX40 co-signaling molecules, which play important roles in T cell-antigen presenting cell interaction, and activate the immune system non-specifically. In this review, I will discuss pathogenic roles of Tax-induced IL-1 in the development of autoimmunity and arthritis in mouse models.

Rheumatoid arthritis (RA) is one of the most serious medical problems worldwide with approximately 1% of the people of all races in the world affected. It is estimated that approximately 700,000 patients are in Japan. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissues in multiple joints that leads to joint destruction, although the etiopathogenesis has not been elucidated completely (*11, 32, 88*).

It is known that people who carry specific major histocompatibility complex (MHC) haplotypes such as HLA-DR4 and DR1 show higher risk for the develop-

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ment of chronic RA than those who carry other MHC molecules, suggesting that antigen-presenting ability to specific pathogenic antigens correlates to susceptibility to the disease (*22, 40, 117, 140*). On the other hand, infectious agents such as viruses, bacteria, and mycoplasmas are also suggested to be involved in the development of arthritis (*130*). In fact, it is well known that bacteria such as mycobacteria and streptococci can induce arthritis in animals when animals are infected with these bacteria (*15, 70*). It is shown that some bacterial components like HSP65 or proteoglycans are immunologically cross-reactive with surface molecules of the synovial cells (*65*). Super antigens in the bacterial components are also suggested to be involved in the development of autoimmunity (*101*).

Involvement of virus infections is also suggested in many studies. It is known that rubella virus infection can cause arthritis transiently (*115*), and Epstein-Barr (EB) virus (*2*) and parvovirus (*122*) are also suspected to be involved. With regard to this, it was shown that amino acid sequences of the viral components from EB virus have sequence homologies with those of DR chains (*21, 110*). Association of arthritis with retrovirus is also reported (*77*). Nishioka *et al.* first reported a patient with chronic inflammatory arthropathy infected with human T cell leukemia virus type I (HTLV-I) (*96*), and, as I will describe later, we found that HTLV-I could induce arthritis in mice (*60*). Other retroviruses such as human immunodeficiency virus (HIV) (*111*) and caprine arthritis and encephalitis virus (CAEV) (*10, 13, 64*) are also known to induce arthritis.

Various animal disease models have been developed to elucidate pathogenesis of the disease and to evaluate efficacy of possible therapeutics. These RA models include spontaneous models, induced models, and gene manipulated mouse models. The MRL/lpr mouse is known as a model for systemic lupus erythematosus (SLE), and some of these mice also develop arthritis spontaneously (*50*). It is known that these mice carry a mutation in the *fas* gene and T cell apoptosis is impaired in these animals (*134*). Collagen-induced arthritis (CIA) is a typical induced arthritis model in which arthritis is induced in rat, rabbit, mouse, or monkey by injecting type II collagen, one of the major components of the joint (*12, 118, 124*). It is known that injection with either mycobacterium cell wall (complete Freund's adjuvant) (*69, 102*), streptococcal cell wall (*15*), peptide glycans (*70*), or muramyl dipeptide (*71*) also induces acute joint inflammation. It has been shown that molecular mimicry is present between cartilage antigens and a mycobacterium antigen (heat shock protein: HSP65) (*129*) or a streptococcal cell wall antigen (*128*).

Several RA models are produced using transgenic techniques. The transgenic mice carrying a modified human *tumor necrosis factor (TNF)-α* gene under its own promoter, which produces very stable TNF-α mRNA, develop arthritis by 4 weeks of age (*66*). Similar phenotypes were observed in mice lacking TNF AU-rich elements which regulate TNF-α mRNA destabilization (*72*). Since this arthritis develops in the $RAG^{-/-}$ background, the involvement of innate and/or stromal mechanism rather than the autoimmune mechanism is suggested (*72*). As I will describe later, transgenic mice carrying a human *interleukin (IL)-1α* gene (*95*) and IL-1receptor antagonist-deficient mice (*54*) develop arthritis, indicating that IL-1 also plays important roles in development of the symptoms. Furthermore, we also developed a transgenic (Tg) arthritis model which carries the HTLV-I *tax* gene. Although each model has its own merits, the HTLV-I Tg mouse model is important, because epidemiological studies

suggest that this virus actually causes RA in humans (*26, 97*).

In spite of enormous efforts, the etiopathogenesis of RA remains mostly unknown. Especially, it is critically important to elucidate mechanisms of the development of autoimmunity, because autoimmunity triggers the development of the disease. In this review, I will discuss pathogenic roles of cytokines, especially of IL-1, in the development of arthritis and autoimmunity caused by HTLV-I.

HTLV-I Tg Mouse Model

HTLV-I is known as the causative agent of adult T cell leukemia (*121, 137*). This virus encodes a transcriptional transactivator, Tax, in the *pX* region, that transactivates transcription from the cognate viral promoter through the 21 bp enhancer (*138*). It was shown that Tax also activates many cellular genes (*137*) including those for cytokines such as IL-1, IL-2, IL-6, nerve growth factor (NGF), transforming growth factor (TGF)-β, and granulocyte macrophage colony-stimulating factor (GM-CSF) (*67, 82, 86, 114, 133*), cytokine receptors such as α-subunit of IL-2 receptor (*42, 56*), and the immediate early transcriptional factors c-fos and c-jun (*34, 35, 59*) through activation of enhancers like the NF-κB-dependent enhancers or serum responsive elements (*138*).

Previously, we found that Tg mice carrying the HTLV-1 *env-pX* region with its own long terminal repeat (LTR) promoter (HTLV-I Tg mice) developed chronic inflammatory polyarthropathy at a high incidence (*60*). The arthritis develops spontaneously in multiple joints as early as 4 weeks of age, and at 3 months of age, 60% (BALB/cAn background) of the mice are affected. The histopathology is very similar to that of RA in humans, showing marked synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissues (*135*). These mice develop autoimmunity with elevated levels of antibodies against immunoglobulin (Ig) G, type II collagen, and HSPs (*58*), and show IgG hyper-gammaglobulinemia in which agalactosylated forms of the Ig carbohydrate chains increase (*27*).

We also produced transgenic mice that expressed the *tax* gene alone under the control of either its own LTR or the CD4 enhancer/promoter and found that both of these mice developed RA-like inflammatory arthropathy similar to HTLV-I Tg mice that carry the LTR-*env-pX*-LTR region, indicating that the *tax* gene is arthritogenic (*49*).

The incidence of arthritis greatly differs among mouse strains (*57*). When HTLV-I Tg mice were backcrossed with different inbred strains, the incidence of arthritis was: 64% and 72% in BALB/cAn ($H\text{-}2^d$), 25% and 46% in C3H/HeN ($H\text{-}2^k$), and 0% and 2% in C57BL/6J ($H\text{-}2^b$) background at 3- and 6-months of age, respectively. Rheumatoid factor (antibodies against IgG) levels in the serum correlated with the susceptibility to the disease, whereas *IL-1β* and *MHC* gene expression were similarly elevated in all these strains, suggesting involvement of immune regulatory genes in this strain difference. However, introduction of the $H\text{-}2^d$ locus into C57BL/6J *pX*-Tg mice did not increase the incidence and substitution of the BALB/cAn *H-2* locus with the $H\text{-}2^b$ did not decrease it. The results indicate that the *H-2* locus is not the major determinant of the disease. Since involvement of multiple genes in RA is also suggested in humans, identification of these susceptibility genes may provide useful information to identify susceptible genes in humans.

Autoimmune pathogenesis was suggested in this model because high levels of autoantibodies were detected in the serum, oligoclonal T cells were accumulated in the joints, and the disease onset was suppressed in athymic *nu/nu* mice (*49, 57, 58, 76*). Furthermore, when bone-marrow (BM) cells from HTLV-I Tg mice were transferred into irradiated non-Tg mice, arthritis developed in these mice (*113*). In contrast, arthritis in HTLV-I Tg mice was completely suppressed by the transplantation of non-Tg BM cells, indicating that the disease is not caused by the abnormal education of T cells in the periphery but by the disorder in the stem cells.

The T cell receptor (TCR) β-chain variable region (Vβ) repertoires in the lymphatic organs are normal in transgenic mice, however, specific Vβ positive T cells are expanded oligoclonally in the affected joints, suggesting that specific antigens, but not superantigens, were involved in the expansion of these T cells (*76*). These expanded T cells had the same TCRs as those of lymph node T cells reactive to type II collagen. Moreover, these mice became susceptible to CIA even on the C3H/HeN background. These observations indicate that endogenous type II collagen is one of the arthritogenic antigens in the joint, suggesting a tolerance break to this antigen in HTLV-I Tg mice.

We found that both TCR Vβ11 and Vβ12 positive populations were greatly reduced in the spleen and lymph modes (LNs) in transgenic mice as in non-transgenic C3H/HeN mice by endogenous mouse mammary tumor virus (MMTV) superantigens, indicating that negative selection in the thymus proceeds normally (*76*). On the other hand, we found that T cells from HTLV-I Tg mice were refractory against anti-Fas antibody treatment (*68*). This finding suggests that this defect of T cells may be involved in the development of autoimmunity in these mice, because Fas-mediated apoptosis of T cells is believed to be important in eliminating autoreactive T cells in the periphery (*90*). In support of this idea, we showed that the incidence increased when these HTLV-I Tg mice were crossed with *lpr/lpr* mice, while it decreased when they were crossed with *fas* Tg mice (*57*). These observations suggest that aberration of Fas-mediated apoptosis of peripheral lymphocytes rather than negative selection in the thymus is involved in the development of autoimmunity in HTLV-I Tg mice. Furthermore, they suggest that administration of Fas ligand or anti-Fas antibody may be effective for the treatment of arthritis.

It seems difficult, however, to explain the development of autoimmunity solely by the defect of Fas-mediated apoptosis, because even *lpr/lpr* mice develop arthritis only on a specific genetic background and never develop arthritis on the C3H/HeN background on which HTLV-I Tg mice develop arthritis. In this context, the finding that Vβ12 positive T cells reactive with type II collagen expanded oligo-clonally in the affected joints (*76*) was interesting, because it suggested that T cells in the affected joints had recovered from the anergic state caused by MMTV superantigens. This tolerance break at the local sites could explain the development of autoimmunity in these HTLV-I Tg mice.

Suppression of the Development of Arthritis in IL-1-deficient Mice

It is remarkable that various proinflammatory cytokine genes including IL-1α, IL-1β, IL-2, IL-6, TNF-α and interferon (IFN)-γ are activated in the transgenic joints (*58*), probably due to the transcriptional transactivating activity of Tax. Among

them, IL-1 is especially interesting, because augmentation of *IL-1* gene expression in the joints of RA patients is also reported by many investigators (*31*). Production of IL-1 is also reported in animal models such as antigen-induced arthritis in the rabbit and CIA in the mouse (*81*). Furthermore, correlation of plasma IL-1 levels with disease activity is reported in rheumatoid arthritis (*25*) and a genetic association between juvenile rheumatoid arthritis and an IL-1α polymorphism is reported (*84*).

Arthritogenic activity of IL-1 is demonstrated by the facts that injection of purified rIL-1 into rabbit knee joints induces the accumulation of polymorphonuclear and mononuclear leukocytes in the joint space and the loss of proteoglycan from the articular cartilage (*103*); continuous infusion of human rIL-1α into the rabbit knee joint induces arthritis (*28*), and mouse rIL-1 induces an acute exacerbation of arthritis in rat joints previously injected by group A streptococcal peptidoglycan-polysaccharide (*119*). These observations suggest involvement of IL-1 in the pathogenesis of RA.

Then, we examined the effect of IL-1 deficiency on the development of arthritis in HTLV-I Tg mice and found that development of arthritis was markedly suppressed in IL-1-deficient mice (*112*). The incidence of arthritis was 60% and 80% at 3 months and 6 months of age, respectively, in wild-type mice. In contrast, that in IL-1α/β$^{-/-}$ mice is only 10% at 3 months and 30% at 6 months of age. However, the severity score of the affected mice was as high as that in wild-type mice, indicating that IL-1 plays an important role in the initial phase of the disease, but that this pathologic role can be substituted by some other factors in the later stage of the disease.

We also found that the development of CIA was severely suppressed in IL-1-deficient mice: 68% of wild-type mice developed arthritis 10 weeks after type II collagen immunization, whereas none (0%) of the IL-1α/β$^{-/-}$ mice developed arthritis (*112*). These results indicate that IL-1 also plays a crucial function in the development of arthritis in this model.

Consistently with our results, it was reported that treatment with anti-IL-1α/β as well as anti-IL-1β antibody ameliorated CIA in mice (*63, 127*). However, in these reports, anti-IL-1α antibody treatment showed no significant protection. It is also reported that local expression of IL-1Ra protein can prevent murine CIA (*4, 126*).

Roles of IL-1 in the Development of Arthritis

It is known that IL-1 is produced by various types of cells, including macrophages, monocytes, and synovial lining cells, and induces inflammation by activating synovial cells, endothelial cells, lymphocytes and macrophages to produce various chemokines, cytokines, and inflammatory mediators (*29*). These inflammatory mediators include IL-1 itself, IL-6, TNF-α, IL-8 and cyclooxygenase (COX)-2, and cause infiltration of inflammatory cells into inflammatory sites, increase the permeability of the blood vessels, and induce fever (*14, 17, 123*). Furthermore, IL-1 promotes synovial cell growth and activates synovial cells and osteoclasts to produce metalloproteases and collagenases that cause erosion of the bone and cartilage of joints (*16*). Thus, it is possible that these activities of IL-1 are involved in the development of arthritis at the effector phase. However, since the severity score of the affected mice in IL-1-deficient HTLV-I Tg mice was similar to wild-type mice, it was suggested that these activities of IL-1 were not necessarily required at the elicitation phase for the

development of inflammation in the HTLV-I Tg model (*112*).

Then, we analyzed the effects of IL-1 deficiency on the immune system. In the immune system, IL-1 is known to activate lymphocytes, monocytes, macrophages and NK cell, although the mechanisms have not been elucidated (*18, 19*). When mice were immunized with protein antigens together with IL-1, serum antibody production was enhanced, suggesting that IL-1 has an adjuvant effect (*107, 116*).

Antibody production after immunization with type II collagen in the presence of complete Freund's adjuvant (CFA) in the CIA model was normal in IL-1$\alpha/\beta^{-/-}$ mice, while spontaneous autoantibody production against type II collagen was suppressed in IL-1$\alpha/\beta^{-/-}$ HTLV-I Tg mice (*112*). Thus, IL-1 is required for the development of autoantibodies in HTLV-I Tg mice, whereas IL-1 seems not to be required for the production of antibodies against type II collagen in the CIA model. We reported previously that adjuvant abrogated the effects of IL-1 deficiency, because various cytokines such as TNF-α or IL-6, which have similar biological activities as IL-1, were induced by the treatment (*91*). Nonetheless, CIA was severely suppressed in the IL-1-deficient mice. Thus, the function of IL-1 in enhancing antibody production does not seem crucial for the development of arthritis. On the other hand, T cell proliferating response against type II collagen was greatly reduced in both CIA and HTLV-I Tg models, suggesting requirement of IL-1 in T cell activation (*112*). The role of IL-1 in the immune system was further analyzed using IL-1Ra$^{-/-}$ mice.

Development of Autoimmune Arthritis in IL-1Ra Knockout Mouse

IL-1Ra binds IL-1 receptors without exerting agonistic activity, and competes for the binding of both IL-1α and IL-1β (*8, 51*). We have recently generated IL-1Ra-deficient mice and showed that these mice developed chronic inflammatory arthropathy spontaneously (*54*). The incidence of arthritis differs among different genetic backgrounds; the incidence is high in the BALB/c background, but low in the C57BL/6J background, the same tendency that is observed in HTLV-I-induced arthritis. The arthritis starts to develop at 5 weeks of age, and at 12 weeks of age almost all the BALB/c background mice suffered from arthritis. The histopathology of the lesion shows marked synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissues, closely resembling that of RA in humans. Activation of osteoclasts is obvious in the pannus, and infiltration of inflammatory cells mostly consisting of neutrophils are detected in the pannus and the synovial space. Fibrin clots are also detected in the synovial space.

Interestingly, total immunoglobulin levels as well as autoantibody levels against immunoglobulin, type II collagen and dsDNA are elevated in these mice (*54*). When *Rag2* gene in the IL-1Ra$^{-/-}$ mice was deleted, the development of arthritis was completely suppressed (Horai and Iwakura, unpublished observation). Furthermore, when T cells from IL-1Ra knockout mice were transferred to *nu/nu* mice, these mice developed arthritis (Horai and Iwakura, unpublished observation). These observations clearly indicate that excess IL-1 signal on T cells causes autoimmunity, resulting in joint specific inflammation and bone destruction.

Recently, it was reported that transgenic mice carrying the human *IL-1α* gene on the C3H/HeN background developed arthritis spontaneously (*95*). The authors suggested, however, that macrophages and polymorphonuclear neutrophils were directly

involved in the joint destruction, although they had not examined the possibility that autoimmunity is involved in the pathogenesis.

We then asked why IL-1Ra deficiency causes autoimmunity. We found that IL-1α, IL-1β and IL-1Ra were all constitutively expressed under the physiological conditions in the joints of normal wild-type mice, and the expression of IL-1β, but not IL-1α, increased 2 to 3 times in IL-1Ra knockout mice compared to wild-type mice (*54*). The expression of the other inflammatory cytokines TNF-α and IL-6 was also elevated, indicating regulatory roles of IL-1Ra in the cytokine network. It is thought that this elevation of cytokine expression in IL-1Ra knockout mice is caused by excess IL-1 signal, because IL-1 can induce IL-6, TNF-α, and IL-1 itself (*17, 53*). Thus, in the absence of IL-1Ra, physiological levels of IL-1 induce pathogenic IL-1 responses resulting in the development of autoimmunity. These results have been the first to suggest a link between excessive IL-1 signal and autoimmunity, and that the balance between IL-1 and IL-1Ra is crucial for the homeostasis of the immune system.

T Cell Sensitization with IL-1 through Induction of CD40 Ligand (L) and OX40

Since it is suggested that IL-1 plays a crucial role in activating the immune system, we then analyzed the mechanism. For this, we investigated roles of IL-1 in T cell-dependent (TD) antibody production using gene-targeted mice. Both primary and secondary antibody production against sheep red blood cells (SRBC) were significantly reduced in IL-1α/β$^{-/-}$ mice, and were enhanced in IL-1Ra$^{-/-}$ mice (*91, 92*). The intrinsic functions of B cells, T cells, and antigen-presenting cells (APCs) were normal. However, proliferative response and cytokine production of T cells against SRBC through the interaction with APCs were markedly impaired when APCs from IL-1α/β$^{-/-}$ mice were used, and enhanced when those from IL-1Ra$^{-/-}$ mice were used. These results indicate that IL-1 produced by APCs promotes the antigen-specific T cell helper function and promotes TD antibody production.

Furthermore, we found that IL-1$^{-/-}$ APCs did not fully activate T cells from DO11.10 mouse, an ovalbumin (OVA)-specific T cell receptor Tg mouse, upon stimulation with OVA, while IL-1Ra$^{-/-}$ APCs were more efficient to activate T cells (*91*). These observations indicate that IL-1 promotes T cell-priming.

In analyzing molecules involved in the interaction between T cell and APC, we found that the expression of co-signaling molecules, CD40L and OX40, on T cells was reduced upon interaction with IL-1$^{-/-}$ APCs and increased upon interaction with IL-1Ra$^{-/-}$ APCs. The expression of these molecules on T cells was induced directly with recombinant IL-1, although the preceding induction of IL-1R on T cells through activation of TCR was required in naive T cells. Furthermore, the reduced antibody production in IL-1$^{-/-}$ mice was recovered by the treatment with agonistic anti-CD40 monoclonal antibody (mAb) both *in vitro* and *in vivo*. These results indicate that IL-1 activates T cells by inducing CD40L and OX40 expression on T cells.

Roles of the CD40L/CD40 and OX40L/OX40 System in the Immune System

The CD40L (CD154) is a type II membrane glycoprotein that is expressed transiently on CD4^{+} helper T cells after activation. Interactions between CD40L and CD40, its mitogenic receptor on B cells, provide an essential signal for the induction

of B cell activation and Ig production. Abnormal CD40L expression causes the Ig class switch defects observed in the X-linked hyper-IgM immunodeficiency syndrome which is characterized by elevated levels of IgM and low levels of IgA, IgG, and IgE, the absence of germinal centers, and the inability to mount TD humoral response (*20, 74, 106*). CD40 is expressed by a large variety of cell types other than B cells, and these include dendritic cells, follicular dendritic cells, monocytes, macrophages, mast cells, fibroblasts, and endothelial cells (*44*). Thus, the CD40-CD40L interaction is also important to produce inflammatory cytokines, chemokines, and IL-12 from APCs, to induce co-signaling molecules, and to activate macrophages, natural killer (NK) cells, and endothelial cells (*30*). Importantly, it is shown that CD40 signal can also induce another co-signaling molecule, OX40L, on APCs (*89*). As a result, CD4 T cell priming is enhanced through this interaction, and T cell-priming to both protein antigens and alloantigens is impaired in CD40L-deficient mice (*45*). This interaction is also important to enhance CD4 and CD8 T cell clonal expansion, and to protect from the death of the activated T cells in the periphery (*83*).

The role of CD40-CD40L signaling in the development of autoimmunity such as collagen-induced arthritis, experimental autoimmune encephalitis (EAE), lupus nephritis, colitis, and oophoritis has been demonstrated in mouse models (*23, 37, 46, 87*). Treatment of mice with anti-CD40L antibodies blocks development of diseases in these models. It is reported that insufficiency of CD40L-CD40 interaction is involved in neonatal transplantation tolerance (*33*), and excess CD40 signal in murine epidermis in CD40L transgenic mice causes chronic skin inflammation and systemic autoimmunity (*85*).

OX40 (CD134) is another important co-signaling molecule that is involved in the activation of T cells and APCs (*79*). OX40 and OX40L is a member of the TNF receptor (R) and TNF families, and is expressed on activated T cells and APCs (*6, 80*), respectively. OX40 is not constitutively expressed, and is induced on naive T cells after stimulation with antigens or infection with HTLV-I (*52*). OX40 is expressed most strongly on CD4 T cell blasts in the T cell zone (*24*). OX40L is also inducible and the CD40 signal is important to induce this molecule on APCs (*89*). OX40L is also expressed on HTLV-I-infected human leukemic T cells (*6*). Several groups have shown that OX40L expressed on APCs can provide costimulation to CD4 T cells (*1, 7, 36, 39, 41*), and promotes T cell survival through induction of Bcl-xL and Bcl-2 (*108*). Activation of CD8 T cells is also reported (*132*), although $OX40^{-/-}$ mice retain primary and memory cytotoxic T cell response after infection with lymphocytic choriomeningitis virus (LCMV) and influenza virus (*73*).

OX40 stimulation of B cells increases immunoglobulin heavy chain mRNA levels and immunoglobulin secretion (*120*), and the OX40-OX40L interaction is necessary for the differentiation of activated B cells into highly Ig-producing cells. It was shown that T cell-priming was impaired in $OX40L^{-/-}$ mice accompanied by a concomitant reduction of both Th1 and Th2 cytokine production (*89*). Antibody production against keyhole limpet hemocyanin (KLH) and cytotoxic T lymphocyte (CTL) induction were suppressed in those mice, although other investigators reported that serum antigen-specific Ig levels were similar to wild-type mice when $OX40^{-/-}$ or $OX40L^{-/-}$ mice were immunized with various TD antigens (*9, 73, 105*).

$OX40^{-/-}$ mice exhibit an impaired contact hypersensitivity response due to defects in T cell-priming and cytokine production (*9*). It is also reported that the

OX40-OX40L system is involved in many diseases such as EAE (*94, 98*), graft-versus-host disease (GVHD) (*75*), colitis (*80*) and asthma (*62*). Thus, OX40-OX40L interaction seems to regulate not only antibody production but also Th1-dependent cellular responses as well as CTL responses.

The Role of IL-1 in the Development of Autoimmunity and Arthritis

Collectively, the role of IL-1 in T cell and APC activation by antigenic stimulation is considered to be as follows. First, when APCs encounter an antigen, these cells produce IL-1 in response to the antigenic stimulation. On the other hand, IL-1R is induced on T cells by the TCR signal upon interaction with APCs. Then, APC-derived IL-1 activates T cells to induce CD40L and OX40, and the CD40L activates APCs and B cells by interacting with constitutively expressed CD40 molecules, leading to the activation of these cells to express OX40L on the surface. This OX40L again activates T cells that are primed to express OX40 on the surface. These T cells include both CD4 T cells and CD8 T cells, and activate both cellular and humoral immunity by producing Th1 as well as Th2 cytokines (Fig. 1).

Thus, over-expression of CD40L and OX40 can explain the development of autoimmunity in animals in which excess IL-1 signal is generated. Actually, our findings that CD40L and OX40 expression on T cells are augmented in HTLV-I Tg mice and that this enhanced expression is normalized when IL-1 is deleted suggest that overproduction of IL-1 causes autoimmunity through induction of CD40L and OX40 molecules on T cells (*112*). Arthritis development in IL-1Ra$^{-/-}$ mice was also

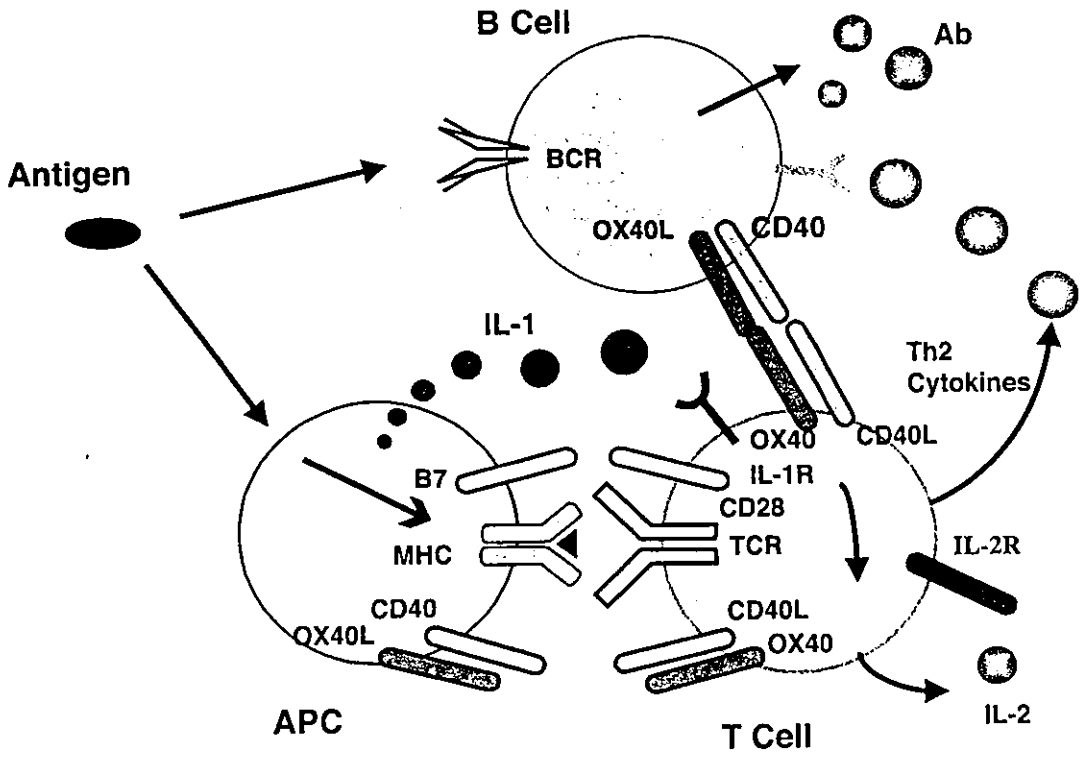

FIG. 1. Activation of the acquired immune system with IL-1

ameliorated when mice were treated with anti-OX40L or anti-CD40L antibodies, suggesting that overexpression of these molecules is responsible for the autoimmunity (Horai *et al.*, unpublished observation). With regard to this, it was reported recently that anti-OX40 antibody treatment was effective to treat CIA model mice (*139*). However, the mechanism seems to be different: development of autoimmunity is suppressed by the treatment in HTLV-I Tg or IL-1Ra$^{-/-}$ mouse models, whereas in the CIA model, T cell-APC interaction upon immunization with collagen is just inhibited.

Involvement of IL-1 in the development of autoimmunity is further supported by the fact that IL-1Ra$^{-/-}$ mice developed CIA even on the C57BL/6J background. Since CIA is never developed in C57BL/6J wild-type mice, these observations suggest that excess IL-1 signal makes the immune system hypersensitive to antigenic challenge (Horai and Saijo, unpublished observation). We have also shown that C3H/HeN mice which are usually resistant to CIA became susceptible to the treatment with type II collagen when they were introduced with HTLV-I *tax* gene (*112*). Consistently with our notion, it is reported that exogenous IL-1 prevents tolerance induction by staphylococcal enterotoxin B (SEB) (*93*), and that signals from an agonistic antibody against OX40 can break an existing state of tolerance in the CD4 T cell compartment (*5*).

It is known that T cells become anergic when these cells encounter antigens without CD28 stimulation (*99*). However, transgenic expression of the B7-1 (CD80) on the islets of Langerhans is not sufficient to abolish the *in vivo* tolerance to islet antigen, and coexpression of the B7-1 and high levels of the class II MHC antigen I-E or TNF-α can induce autoimmune destruction of the β cells of the pancreas (*47, 48*). Our data indicate that IL-1 can activate T cells in the absence of CD28 stimulation, because only the signals from CD3 and IL-1R are enough to activate T cells (*91*). It should be noted that the IL-1 signaling pathway and the CD28 signaling pathway are independent, because CTLA4, a potent inhibitor of the CD28 pathway, could not inhibit IL-1-mediated T cell activation (*91*). Therefore, IL-1 can activate self-reactive T cells which are made anergic in the periphery due to the lack of CD28 costimulation. Since TNF-α can induce IL-1 *in vivo*, the action of TNF-α in abolishing tolerance may actually be mediated by TNF-α-induced IL-1.

Thus, in HTLV-I Tg mice high levels of expression of Tax in synovial cells are thought to directly induce production of inflammatory cytokines, especially of IL-1. These cytokines activate the immune system through induction with CD40L and OX40 on T cells, leading to a tolerance break against synovial antigens. Probably, prolonged survival of T cells due to the reduced sensitivity to Fas-mediated apoptotic signal may also be involved in the development of autoimmunity. This autoimmunity induces production of various inflammatory cytokines, chemokines, inflammatory mediators, and autoantibodies, that exaggerate inflammation. At the same time, these inflammatory cytokines activate synovial cells and/or T cells to produce osteoclast differentiation factor (ODF, or receptor activator of NF-κB ligand: RANKL) (*136*), resulting in the activation of osteoclasts (Fig. 2).

Consistently with our observations, previous studies have also suggested that HTLV-I may be involved in immunological disorders. For example, patients with SLE are found to be carriers of HTLV-I at higher rates than the general population (*104*). HTLV-I has also been suspected of being involved in Sjögren's syndrome

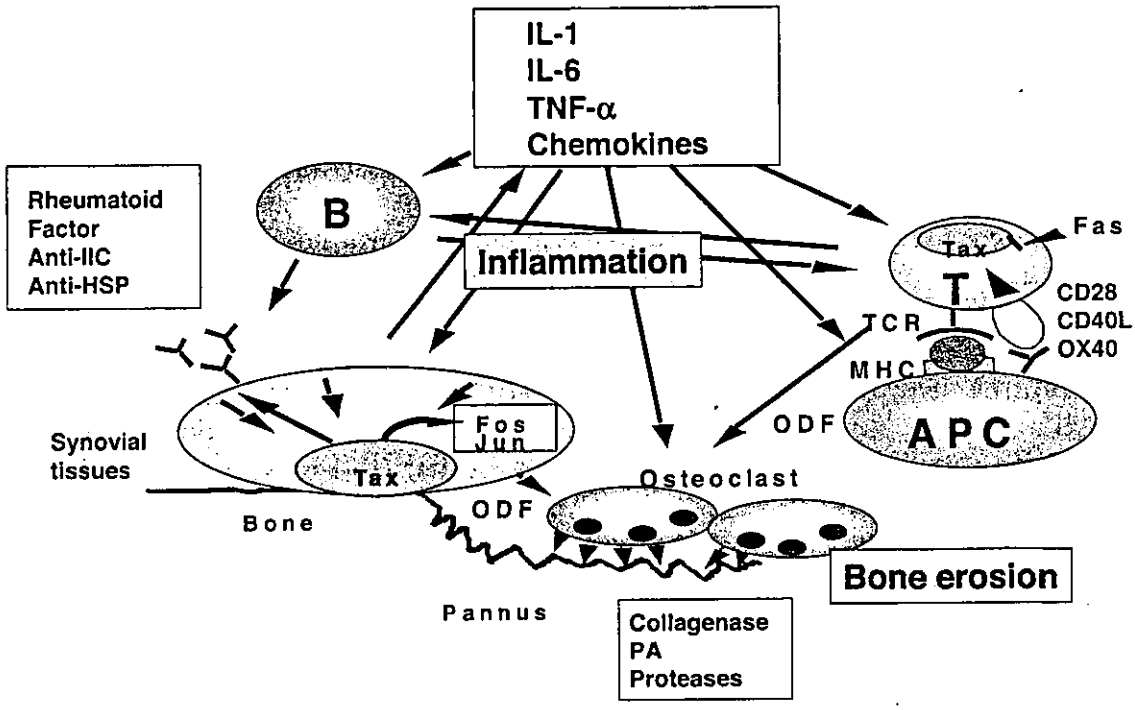

FIG. 2. Pathogenesis of arthritis caused by the HTLV-I *tax* gene

(*131*), and it was shown that transgenic mice carrying the *tax* gene developed an exocrinopathy resembling this syndrome, although there was no data on immunological status reported in those studies (*43*). Elevated immunoglobulin levels (*100*) and increased numbers of autoreactive T cells (*125*) have been reported in patients with HTLV-I-associated myelopathy (HAM), also known as tropical spastic paraparesis (TSP), and augmented immune reactivity against HTLV-I antigens has been implicated in the pathogenesis (*38, 61, 100, 125*). Abnormal immune response against EB virus was reported in patients with adult T cell leukemia (ATL) and healthy HTLV-I carriers (*55*). Other studies have suggested that decreased immune surveillance in HTLV-I carriers may be responsible for their tendency to develop various types of tumors at higher rates than uninfected people (*3*). These observations, however, have not as yet proved a direct pathogenic role of HTLV-I in these immunological disorders. The results presented in the present paper strongly suggest direct involvement of HTLV-I in the induction of these immunological disorders.

It should be noted that the antigenicity of the pathogens is not necessarily cross-reactive with the autoantigens of the host to break the tolerance. In fact, no immunological cross-reactivity was reported between HTLV-I and synovial tissues. The fact that IL-1Ra deficiency causes autoimmunity clearly indicates that excess IL-1 signal is enough to cause autoimmunity. In support of this notion, an enhancing effect of complete Freund's adjuvant (CFA) or inflammation on the development of autoimmunity upon immunization with cross-reactive molecules was reported (*78, 109*). Other than microbial infections, either inflammation caused by mechanical injury or immune reaction, or invasion of a transcriptional transactivator that can activate the *IL-1* genes, or genetic disorder of the IL-1 or its regulatory genes may also cause

overproduction or excess signal of this cytokine. Frequent development of arthritis after microbial infections such as mycobacteria or streptococci may be explained in part by this excess-IL-1 mechanism.

Although IL-1 was first discovered as a major mediator of inflammation, it has gradually become evident that this cytokine has numerous functions related to host defense mechanisms, not only regulating the immune system, but also the areas of the neuronal and endocrine systems that interface with the immune system (*17, 18, 123*). Actually, we showed that IL-1 was induced in the brain upon inflammation and associated fever development was not observed in IL-$1^{-/-}$ mice and exaggerated in IL-$1Ra^{-/-}$ mice, indicating that IL-1 plays a crucial role in the development of fever by acting on the neuronal system (*53*). Furthermore, we showed that IL-1 is also involved in the regulation of glucocorticoid secretion through the hypothalamus-pituitary-adrenal cortex axis (*53*). Thus, it is suggested that IL-1 plays critically important roles in the development of RA, not only inducing inflammatory responses but also activating the immune system, the neuronal system and the endocrine system. The control of the production and the activity of this cytokine should be critically important for the treatment of RA.

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