Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 8222–8226, July 1998
Immunology

Interleukin 6 plays a key role in the development of antigen-induced arthritis

(rheumatoid arthritis/proinflammatory cytokine)

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Contributed by Tadamitsu Kishimoto, April 20, 1998

ABSTRACT To investigate the direct role of interleukin (IL) 6 in the development of rheumatoid arthritis, IL-6-deficient (IL-6 −/−) mice were backcrossed for eight generations into C57BL/6 mice, a strain of mice with a genetic background of susceptibility for antigen-induced arthritis (AIA). Both histological and immunological comparisons were made between IL-6-deficient (IL-6 −/−) mice and wild-type (IL-6 +/+) littermates after the induction of AIA. Although all IL-6 +/+ mice developed severe arthritis, only mild arthritis was observed in IL-6 −/− mice. Safranin O staining demonstrated that articular cartilage was well preserved in IL-6 −/− mice, whereas it was destroyed completely in IL-6 +/+ mice. In addition, comparable mRNA expression for both IL-1β and tumor necrosis factor α, but not for IL-6, was detected in the inflamed joints of IL-6 −/− mice, suggesting that IL-6 may play a more crucial role in cartilage destruction than either IL-1β or tumor necrosis factor α. In immunological comparisons, both antigen-specific *in vitro* proliferative response in lymph node cells and *in vivo* antibody production were elicited in IL-6 −/− mice, but they were reduced to less than half of that found in IL-6 +/+ mice. Lymph node cells of IL-6 −/− mice produced many more Th2 cytokines than did IL-6 +/+ mice with either antigen-specific or nonspecific stimulation in *in vitro* culture. Taken together, these results indicate that IL-6 may play a key role in the development of AIA at the inductive as well as the effector phase, and the blockade of IL-6 is possibly beneficial in the treatment of rheumatoid arthritis.

Rheumatoid arthritis (RA) is one of the major immune-mediated diseases. The cause of RA remains unknown, but several hypotheses, involving an autoimmune mechanism (1), superantigen-driven disease (2), and infectious stimulus (3), have been postulated. Whatever the cause of RA, recent cumulative evidence suggests that cytokines are important mediators for the pathology of RA (4). The cytokines, especially so-called proinflammatory cytokines, such as interleukin (IL) 1, tumor necrosis factor (TNF), and IL-6, play a pivotal role in the pathology of RA. The pathology of RA consists of: (*i*) acute and chronic inflammation, (*ii*) cell proliferation, and (*iii*) tissue destruction/fibrosis. Biologic actions of these proinflammatory cytokines appear to contribute to all three aspects of RA pathology. In addition, recent progress in the field of cytokine research has led to the idea that these proinflammatory cytokines might be optimal therapeutic targets for RA. In fact, recent clinical trials of anticytokine therapy for RA (5–9) encouraged this idea. However, the roles of the individual cytokines for the pathology of RA have not been fully dissected and understood. Particularly, the idea of IL-6's direct involvement in joint destruction is still less convincing and more controversial than that of the involvement of IL-1 or TNF (10–16). One of the possible reasons is that given the high levels of IL-6 at inflamed joints it is difficult to approach the role of IL-6 in animal models with the use of neutralizing antibodies, as incomplete blocking can hardly be excluded.

Antigen-induced arthritis (AIA) is an animal model for arthritis initially induced in rabbits and extended into mice (17). AIA is T cell-dependent immunological arthritis, and its histopathological features closely resemble those of RA. In a mouse with AIA, we can observe most histopathological characteristics of RA, such as marked synovial lining hyperplasia, proliferation of sublining cells, infiltration of inflammatory cells, neovascularization, pannus formation, and articular cartilage destruction. Although AIA is a monoarthritis model, it has the advantage of precise and fair histological comparisons among individual mice.

In this study, to investigate the direct involvement of IL-6 in the pathology of RA, we backcrossed IL-6-deficient (IL-6 −/−) mice for eight generations into C57BL/6 mice, a strain of mice with genetic background of susceptibility for AIA. Both histological and immunological comparisons were made between IL-6 −/− mice and IL-6 +/+ littermates after the induction of AIA.

MATERIALS AND METHODS

Animals. Animal experimentations in this study were performed according to the guidelines of the Animal Experimentation Committee of Osaka University. IL-6 −/− mice were generated as described elsewhere (18). Those IL-6 −/− mice were backcrossed into C57BL/6 mice (Nihon SLC, Shizuoka, Japan) for eight generations. Then, IL-6 −/− mice and IL-6 +/+ littermates were obtained by intercrossing heterozygous (IL-6 +/−) mice for use in this study. All mice were male, 10–12 weeks of age at the time of immunization, and were bred at the Experimental Animal Center of Osaka University Medical School. Mice were housed in filter-top cages under standard pathogen-free conditions.

Induction of AIA. AIA was induced by following the same protocol (17). Briefly, mice were immunized intradermally at the base of the tail and four footpads with 100 μg of methylated BSA (mBSA) (Sigma) emulsified with an equal volume of complete Freund's adjuvant (Difco). They were injected with 2×10^9 *Bordetella pertussis* organism (Kaken Pharmaceutical, Tokyo) i.p. This procedure was repeated on day 7. On day 21, 100 μg of mBSA in 10 μl of saline was injected into the left

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Abbreviations: IL, interleukin; RA, rheumatoid arthritis; AIA, antigen-induced arthritis; TNF, tumor necrosis factor; LNC, lymph node cells; mBSA, methylated BSA; RT, reverse transcriptase.

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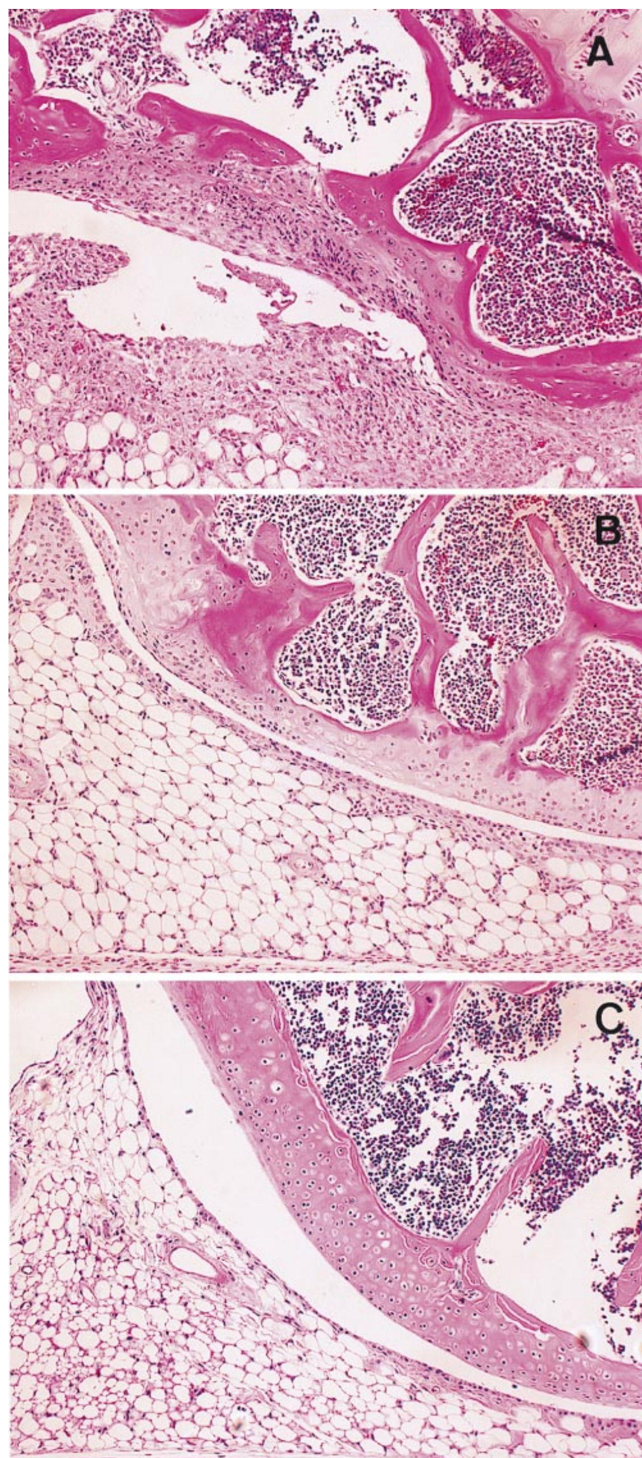

FIG. 1. Representative histopathologies of the knee joints stained with hematoxylin and eosin (×100). Thirty-five days after the first immunization, mice were sacrificed and subjected to histopathological examination. (*A*) Wild-type (IL-6 +/+) mice induced AIA (grade 4); massive cellular infiltration, synovial hyperplasia, neovasculization, and erosion of cartilage and bone were observed in the knee joint. (*B*) IL-6-deficient (IL-6 −/−) mice (grade 2); only limited synovial lining cell hyperplasia was detected. (*C*) Wild-type (IL-6 +/+) mice injected with saline as a control (grade 0); no features of synovitis were detected.

knee joint. As a control, the same volume of saline was injected into the right knee joint.

Histological Examination. Mice were anesthetized with pentobarbital and sacrificed 35 days after the first immunization. The legs were removed, and the knee joints were opened by a transverse incision through the patellar tendon. After fixation in 4% paraformaldehyde in PBS, the legs were decalcified for 10 days with EDTA and embedded in paraffin. The paraffin sections were stained with hematoxylin-eosin, Safranin O-fast green, and tartrate-resistant acid phosphatase. The slides were evaluated histologically by two independent observers, and the gradation of arthritis was scored from 0 to 4 according to the intensity of lining layer hyperplasia, mononuclear cell infiltration, and pannus formation as done previously (17): 0, normal knee joint; 1, normal synovium with occasional mononuclear cells; 2, definite arthritis, a few layers of flat to rounded synovial lining cells and scattered mononuclear infiltrates; 3, clear hyperplasia of the synovium with three or more layers of loosely arranged lining cells and dense infiltration with mononuclear cells; and 4, severe synovitis with pannus and erosions of articular cartilage and subchondral bone.

Table 1. Histological scores for AIA in IL-6-deficient and wild-type mice

Group	Genotype	Mice per group	Histology score of mice at day 35					
			0	1	2	3	4	Mean ± SE
I	IL-6 +/+	10	—	—	—	2	8	3.8 ± 0.18
II	IL-6 −/−	10	—	2	8	—	—	1.8 ± 0.18*

Both groups were immunized in the standard manner and sacrificed 35 days after the first immunization. The histopathologies of the knee joints were scored from 0 to 4 as described in *Materials and Methods.* *, $P < 0.001$, IL-6 −/− compared to IL-6 +/+.

Proliferative Response of the Lymph Node Cells (LNC). Mice were immunized with mBSA twice on days 0 and 7 by the same protocol with the induction of AIA. Fourteen days after immunization with mBSA, mice were sacrificed. The inguinal lymph nodes were removed and prepared for single LNC suspensions. LNC (5×10^5cells/well) were cultured with 0.2 ml of complete culture medium supplemented with 1% mice serum in the absence or presence of either mBSA (100 μg/ml) or ConA (1 μg/ml) in 96-well plates for 72 hr. Cell proliferative response was measured by [^{3}H]thymidine incorporation.

Measurement of Serum Anti-mBSA IgG. Sera were collected on days 0 (before immunization), 7, and 14. Anti-mBSA IgG levels were measured by ELISA as described elsewhere (19). Briefly, 96-well plates were coated overnight with 100 μl of 5 μg/ml mBSA and blocked by incubation with 0.1% goat serum (Kirkegaard & Perry Laboratories) in PBS for 1 hr at 37°C. After washing, a 100-μl aliquot of each mouse serum diluted 5-fold in PBS was applied and incubated for 30 min at 37°C. After another washing, 100 μl of alkaline phosphatase-conjugated horse anti-mouse IgG (Vector Laboratories) was added and incubated for 30 min at 37°C. The plates were washed three times and color development of *p*-nitrophenylphosphate (Kirkegaard & Perry Laboratories) was monitored at 405 nm by an ImmunoReader NJ-2000 (Nihon InterMed, Tokyo).

Th1 (IFNγ and IL-2) and Th2 (IL-4 and IL-10) Cytokines Production in LNC. Mice were immunized with mBSA by the same protocol with the induction of AIA. Fourteen days after immunization with mBSA, mice were sacrificed. The inguinal lymph nodes were removed and prepared for single LNC suspensions. LNC (5×10^5 cells/well) were cultured with 0.2 ml of complete culture medium supplemented with 1% mice serum in the absence or presence of either mBSA (100 μg/ml) or ConA (1 μg/ml) in 96-well plates, and supernatants were collected after 48 hr. IFNγ and IL-4 levels were detected by sandwich ELISA using reagents from PharMingen following the manufacturer's protocol. IL-2 and IL-10 levels were measured with a commercially available ELISA kit (Genzyme). The sensitivity of assay was 5 pg/ml for IFNγ, 0.5 pg/ml for IL-4, 15 pg/ml for IL-2, and 15 pg/ml for IL-10.

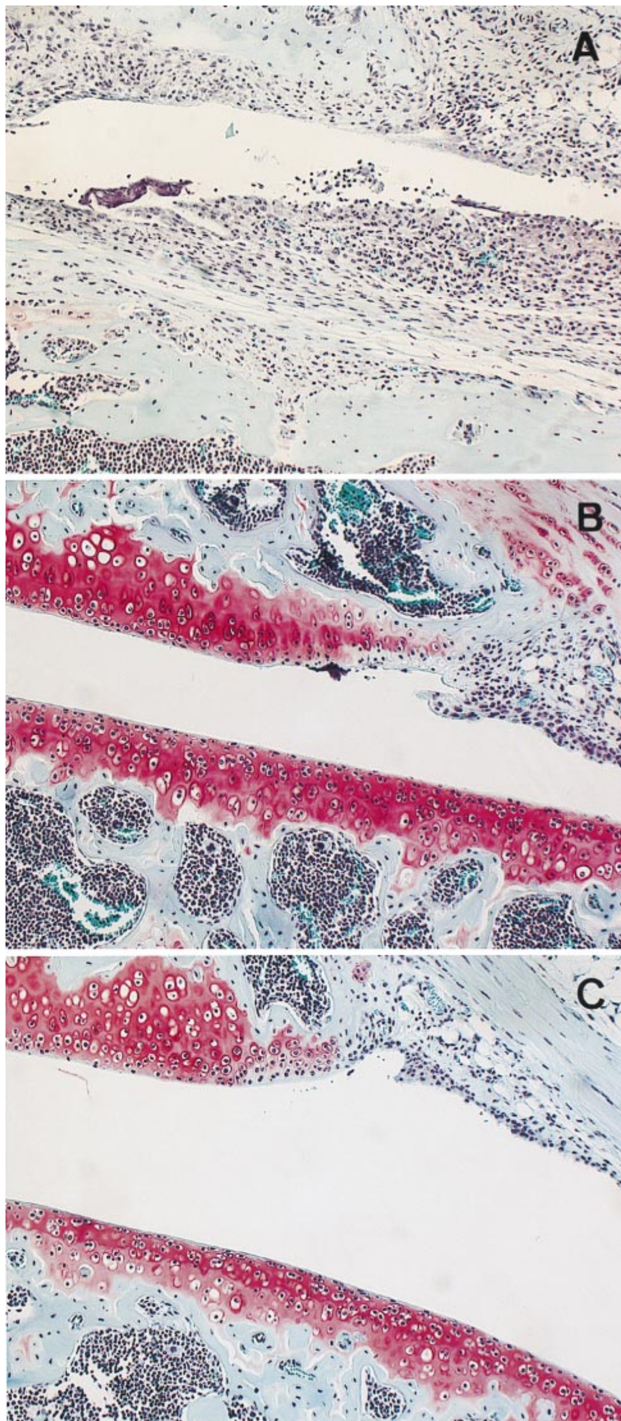

FIG. 2. Femoropatellar sections of the knee joints stained with Safranin O/fast green (×100). (*A*) Wild-type (IL-6 +/+) mice induced AIA; severe synovitis with pannus formation in patella (upper side) and femur (lower side) was observed. Moreover, Safranin O was not stained, indicating complete cartilage destruction. (*B*) IL-6-deficient (IL-6 −/−) mice induced AIA; only limited synovial hyperplasia was detected. Safranin O was stained strongly in patella and femur (red color), indicating cartilage was completely preserved. (*C*) Wild-type (IL-6 +/+) mice injected saline as a control; no features of synovitis were detected, and Safranin O was strongly stained.

Semiquantitative Reverse Transcriptase (RT)–PCR. Synovia of the knee joints from two each of IL-6 −/− and IL-6 +/+ mice after the induction of AIA were examined. Samples from two immunized IL-6+/+ mice injected with saline were used as controls. All mice were sacrificed 25 days after immunization. Each sample was removed and immediately frozen on dry ice, and total RNA was extracted by the acid guanidium thiocyanate-phenol-chloroform method as described elsewhere (20). cDNA was synthesized by RT, and amplification of cDNA by PCR was performed with the primers specific for the individual cytokines (mouse IL-6, TNFα, and IL-1β; CLONTECH), or with β-actin (CLONTECH) as an internal control. cDNA equivalent to 50 ng of total RNA was used for PCR in a final volume of 20 μl containing [α-^{32}P]dCTP (Amersham). The semiquantitative RT-PCR assay was performed as described previously (21, 22). Thirty cycles of PCR were performed under the following conditions: denaturation at 94°C for 30 sec, primer annealing at 60°C for 30 sec, and primer extension at 72°C for 1 min. The PCR products were electrophoresed on a 6% polyacrylamide gel for separation (IL-6, 638 bp; TNFα, 692 bp; IL-1β, 563 bp; β-actin, 540 bp). Moreover, cycle dependency for each primer was confirmed by 24–33 cycles of PCR using 50 ng of RNA samples from lipopolysaccharide-stimulated spleen cells. We also confirmed the dose dependency for each primer by 30-cycle PCR using 0.6–150 ng of the same RNA samples. Quantitative analysis of the PCR products was performed by image analyzer (Fujix BAX1000, Fuji).

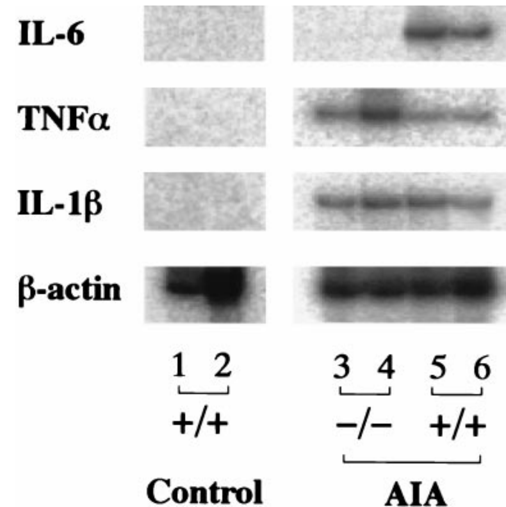

FIG. 3. Semiquantitative RT-PCR analysis for the expression of IL-6, IL-1β, and TNFα in the synovia of knee joints from two each of both IL-6-deficient and wild-type mice at 25 days after immunization. As a control, synovia of saline-injected knee joints from two wild-type mice at day 25 were used. Lanes 1 and 2: wild-type (IL-6 +/+) mice injected saline as a control. Lanes 3 and 4: IL-6-deficient (IL-6 −/−) mice induced AIA. Lanes 5 and 6: wild-type (IL-6 +/+) mice induced AIA.

Statistics. Histologic scores and immunological data are expressed as mean values ± SE. Statistical significance was tested by using the Student's *t* test. Differences were considered statistically significant if the two-sided P-value was <0.05.

RESULTS AND DISCUSSION

AIA is an experimental autoimmune arthritis and closely resembles RA histopathologically (17). Although AIA is a monoarthritis model, it has the advantage for a precise and fair histological comparison. Therefore, we used this model to investigate the role of IL-6 in the pathology of RA, using IL-6 −/− mice on the genetically susceptible C57BL/6 background for eight generations. As a result, all IL-6 +/+ mice developed severe arthritis characterized by marked lining layer hyperplasia, dense mononuclear cell infiltration, neovascularization, pannus formation, and erosions of articular cartilage and bone (Fig. 1*A*) compared with the control mice (IL-6 +/+ mice injected with saline alone) (Fig. 1*C*). These histopathological features closely resembled those of RA. In contrast, only mild

Table 2. Proliferative response of the LNC

Source of LNC		^{3}H-thymidine incorporation, cpm ± SEM		
		None	mBSA	ConA
Immunized	IL-6 (+/+)	622 ± 28	46,526 ± 2,330	45,908 ± 1,638
	IL-6 (−/−)	607 ± 25	13,904 ± 305*	44,827 ± 1,359
Nonimmunized	IL-6 (+/+)	394 ± 29	496 ± 12	9,264 ± 324
	IL-6 (−/−)	377 ± 18	415 ± 19	12,386 ± 1,435

Fourteen days after immunization with mBSA, mice were sacrificed. The inguinal lymph nodes were removed, and single-cell suspensions were prepared. The LNC (5×10^5 cells/well) were cultured with 0.2 ml of complete culture medium in the absence or presence of either mBSA (100 μg/ml) or ConA (1 μg/ml) in 96-well plates for 72 hr. Proliferative response was measured by ^{3}H-thymidine incorporation. *, $P < 0.001$, IL-6 −/− compared to IL-6 +/+.

arthritis characterized by two or three layers of flat synovial lining cells was observed in all IL-6 −/− mice (Fig. 1*B*). The histopathological assessments of arthritis in the individual mice of each group are summarized in Table 1. In IL-6 +/+ mice, eight of 10 were graded as score 4, and the rest were graded as score 3. In contrast, in IL-6 −/− mice, eight of 10 were graded as score 2, and the rest were graded as score 1. The mean histological scores of IL-6 +/+ and IL-6 −/− mice were 3.80 ± 0.18 and 1.80 ± 0.18, respectively. Thus, AIA occurred in IL-6 −/− mice, but its severity was markedly reduced compared to that in IL-6 +/+ mice.

Next, we assessed the cartilage destruction by Safranin O staining. As shown in Fig. 2*A*, no Safranin O staining was detected in the knee joints of IL-6 +/+ mice after the induction of AIA, indicating complete cartilage destruction. In contrast, in IL-6 −/− mice, articular cartilage was stained by Safranin O with the almost same extent as that of the control mice (IL-6 +/+ mice injected with saline alone) (Fig. 2 *B* and *C*), indicating that articular cartilage was well preserved in IL-6 −/− mice. Moreover, the activated multinucleated osteoclasts stained with tartrate-resistant acid phosphatase were observed at erosive lesions with pannus formation in all IL-6 +/+ mice after the induction of AIA, whereas they never were detected in IL-6 −/− mice (data not shown). Tamura *et al.* (12) demonstrated that IL-6 could differentiate bone marrow precursors into activated osteoclasts in the presence of soluble IL-6 receptors, supporting this observation. Cartilage destruction is a major event at the early effector phase of RA, and previous reports have suggested the involvement of proinflammatory cytokines in cartilage destruction. We compared the expressions of proinflammatory cytokines (IL-1β, TNFα, and IL-6) by semiquantitative RT-PCR in the arthritic joints between IL-6 −/− and IL-6 +/+ mice to determine which proinflammatory cytokine is a crucial mediator. As shown in Fig. 3, the expressions of mRNA for all three cytokines were detected in IL-6 +/+ mice after the induction of AIA, and comparable mRNA expressions for IL-1β and TNFα, but not for IL-6, also were detected in IL-6 −/− mice after the induction of AIA. This result indicates that either IL-1β or TNFα is unlikely to be an essential mediator, and IL-6 might play a more crucial role than either IL-1β or TNFα in cartilage destruction of AIA. However, further investigations are needed.

AIA is an immunological arthritis model (17), and IL-6 is well known as an important mediator for immunological reactions (23). To examine antigen-specific immune response in IL-6 −/− mice after the induction of AIA, both mBSA-specific *in vitro* proliferative response in LNC and *in vivo* antibody response were measured in IL-6 −/− mice. As shown in Table 2, significant mBSA-specific proliferative response was observed in the immunized IL-6 −/− mice compared with nonimmunized mice. However, the mBSA-specific proliferative response was reduced to about one-third of the immunized IL-6 +/+ mice. On the other hand, no significant difference was observed in the proliferative response to ConA between immunized IL-6 −/− and IL-6 +/+ mice. Moreover, FACS analysis revealed no difference in either the CD3/B220 or CD4/CD8 ratio between immunized IL-6 −/− and IL-6 +/+ mice (data not shown). These results suggest that the reduction of mBSA-specific proliferative response in the immunized IL-6 −/− mice is not caused by either the universal suppression of T cell response or the changes in the proportion of the subpopulation of lymphocytes. Regarding *in vivo* antigen-specific antibody response (Table 3), significant anti-mBSA IgG also was elicited in the immunized IL-6 −/− mice on days 7 and 14 after the immunization as compared with the nonimmunized mice, whereas the titers were about half of those in the immunized IL-6 +/+ mice. Thus, both antigen-specific *in vitro* proliferative response in LNC and *in vivo* antibody response were elicited in IL-6 −/− mice but reduced to less than half of those in IL-6 +/+ mice.

Recent cumulative evidence has demonstrated the importance of the balance between Th1 and Th2 cytokines in immunological diseases, including RA. In general, RA is thought to be a typical Th1 disease regarding the cytokine profile, and Th2 cytokines play preventive roles in different arthritis models (24, 25). To examine the balance between Th1 and Th2 cytokines in the immunized IL-6 −/− mice, we measured *in vitro* Th1 cytokine (IFNγ and IL-2) and Th2 cytokine (IL-4 and IL-10) production in LNC. As shown in Table 4, LNC of the IL-6 −/− mice produced IFNγ, IL-4, and IL-10 when they were stimulated with either mBSA or ConA. Interestingly, the production of both IL-4 and IL-10 in the LNC of the IL-6 −/− mice was significantly more than that in the LNC of the immunized IL-6 +/+ mice. On the other hand, there is no difference in IFNγ production in LNC between the two groups. The production of IL-2 in LNC of IL-6 −/− mice

Table 3. Titers of antibodies against mBSA in sera

Source of sera		Antibodies against mBSA in sera (mean ± SEM) OD_{405}		
		Before	7 days	14 days
Immunized	IL-6 (+/+)	0.109 ± 0.009	0.750 ± 0.079	1.294 ± 0.102
	IL-6 (−/−)	0.119 ± 0.01	0.476 ± 0.007*	0.616 ± 0.031*
Nonimmunized	IL-6 (+/+)	0.092 ± 0.005	0.108 ± 0.04	0.110 ± 0.01
	IL-6 (−/−)	0.107 ± 0.009	0.110 ± 0.012	0.112 ± 0.013

Sera were collected before and 7 or 14 days after first immunization. The titers of antibodies against mBSA were measured by ELISA. *, $P < 0.005$, IL-6 −/− compared to IL-6 +/+.

Table 4. Th1 and Th2 cytokines production in LNC

Cytokine, pg/ml	Source of LNC	Stimuli		
		None	mBSA	ConA
IFN γ	IL-6 (+/+)	29 ± 6	8,209 ± 564	19,850 ± 590
	IL-6 (−/−)	25 ± 8	7,641 ± 1,237	13,616 ± 770
IL-2	IL-6 (+/+)	<15	58.0 ± 1.0	200 ± 8.1
	IL-6 (−/−)	<15	<15	36 ± 6.9*
IL-4	IL-6 (+/+)	<0.5	1.6 ± 0.1	7.8 ± 0.5
	IL-6 (−/−)	<0.5	7.9 ± 0.7*	42 ± 2.9*
IL-10	IL-6 (+/+)	<15	25 ± 1.0	144 ± 9.1
	IL-6 (−/−)	<15	100 ± 1.9*	771 ± 4.6*

Fourteen days after immunization with mBSA, mice were sacrificed. The inguinal lymph nodes were removed, and single-cell suspensions were prepared. The LNC (5×10^5 cells/well) were cultured with 0.2 ml of complete culture medium supplemented with 1% mice serum in the absence or presence of either mBSA (100 μg/ml) or ConA (1 μg/ml) in 96-well plates for 48 hr. Supernatants were collected, and IFN γ, IL-2, IL-4, and IL-10 levels were determined by ELISA. *, $P < 0.005$, IL-6 −/− compared to Il-6 +/+.

was suppressed significantly on either antigen-specific or nonspecific stimulation compared with that of the IL-6 +/+ mice. Thus LNC of immunized IL-6 −/− preferentially produced Th2 cytokines, but the production of IL-2 was reduced conversely. This finding is not so surprising, because Romani *et al.* (26) demonstrated the preferential induction of Th2 cytokine production after *Candida albicans* infection in IL-6 −/− mice. This finding suggests the balance between Th1 and Th2 cytokine might shift toward the Th2 site in the immunized IL-6 −/− mice, and the induction of Th2 cytokine might contribute partly to the prevention of AIA.

In conclusion, the present study suggests that IL-6 may play a key role in the development of AIA at both the induction phase and the effector phase, and the blockade of IL-6 is possibly beneficial in the treatment of RA.

We thank Mr. Morihana and Mr. Fukuyama for their excellent technical assistance and Miss N. Kameoka for secretarial assistance, This study was supported in part by grants from The Ministry of Education, Science, and Culture of Japan, The Osaka Foundation for Promotion of Clinical Immunology, and Ono Pharmaceutical Company.

1. Gaston, H. (1993) *Ann. Rheum. Dis.* **53,** S17–S21.
2. Paliard, X., West, S. G., Lafferty, J. A., Clements, J. R., Kappler, J. W., Marrack, P. & Kotzin, B. L. (1991) *Science* **253,** 325–329.
3. Phillips, P. E. (1997) *Curr. Opin. Rheumatol.* **9,** 337–344.
4. Feldmann, M., Brennan, F. M. & Maini, R. N. (1996) *Annu. Rev. Immunol.* **14,** 397–440.
5. Wendling, D., Racadot, E. & Wijdenes, J. (1993) *J. Rheumatol.* **20,** 259–262.
6. Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Katsikis, P., Brennan, F. M., Walker, J., Bijl, H., Ghrayeb, J. & Woody, J. N. (1993) *Arthritis Rheum.* **36,** 1681–1690.
7. Campion, G. V., Lebsack, M. E., Lookabaugh, J., Gordon, G. & Catalano, M. (1996) *Arthritis Rheum.* **39,** 1092–1101.
8. Moreland, L. W., Baumgartner, S. W., Schiff, M. H., Tindall, E. A., Fleischmann, R. M., Weaver, A. L., Ettlinger, R. E., Cohen, S., Koopman, W. J., Mohler, K., *et al.* (1997) *N. Engl. J. Med.* **337,** 141–147.
9. Weckmann, A. L. & Varela, J. (1996) *Semin. Arthritis Rheum.* **26,** 539–557.
10. Kotake, S., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T. & Kashiwazaki, S. (1996) *J. Bone Miner. Res.* **11,** 88–95.
11. Romas, E., Udagawa, N., Zhou, H., Tamura, T., Saito, M., Taga, T., Hilton, D. J., Suda, T., Ng, K. W. & Martin, T. J. (1996) *J. Exp. Med.* **183,** 2581–2591.
12. Tamura, T., Udagawa, N., Takahashi, N., Miyaura, C., Tanaka, S., Yamada, W., Koishihara, Y., Ohsugi, Y., Kumaki, K. & Taga, T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11924–11928.
13. Manolagas, S. C. (1995) *Bone* **17,** Suppl., 63S–67S.
14. van de Loo, F. A., Joosten, L. A., van Lent, P. L., Arntz, O. J. & van den Berg, W. B. (1995) *Arthritis Rheum.* **38,** 164–172.
15. van de Loo, F. A. J., Kuiper, S., van Enckevort, F. H. J., Arntz, O. J. & van den Berg, W. B. (1997) *Am. J. Pathol.* **151,** 177–191.
16. Poli, V., Balena, R., Fattori, E., Markatos, A., Yamamoto, M., Tanaka, H., Ciliberto, G., Rodan, G. A. & Costantini, F. (1994) *EMBO J.* **13,** 1189–1196.
17. Brackertz, D., Mitchell, G. F. & Mackay, I. R. (1977) *Arthritis Rheum.* **20,** 841–850.
18. Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H. & Kohler, G. (1994) *Nature (London)* **368,** 339–342.
19. Wooley, P. H., Luthra, H. S., Stuart, J. M. & David, C. S. (1981) *J. Exp. Med.* **154,** 688–700.
20. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
21. Rupp, R. A. W. & Weintraub, H. (1991) *Cell* **65,** 927–937.
22. Okuda, Y., Nakatsuji, Y., Fujimura, H., Esumi, H., Ogura, T., Yanagihara, T. & Sakoda, S. (1995) *J. Neuroimmunol.* **62,** 103–112.
23. Matsuda, T., Suematsu, S., Kawano, M., Yoshizaki, K., Tang, B., Tanabe, O., Nakajima, T., Akira, S., Hirano, T. & Kishimoto, T. (1989) *Ann. N.Y. Acad. Sci.* **557,** 466–476.
24. Girasole, G., Jilka, R. L., Passeri, G., Boswell, S., Border, G., Williams, D. C. & Manolagas, S. C. (1992) *J. Clin. Invest.* **89,** 883–891.
25. Joosten, L. A. B., Lubberts, E., Durez, P., Halsen, M. M. A., Jacobs, M. J., Goldman, M. & van den Berg, W. B. (1997) *Arthritis Rheum.* **40,** 249–260.
26. Romani, L., Mencacci, A., Cenci, E., Spaccapelo, R., Toniatti, C., Puccetti, P., Bistoni, F. & Poli, V. (1996) *J. Exp. Med.* **183,** 1345–1355.