suppressed the development of arthritis (FIG. 2B). The Foxp3-alone transduced group suppressed arthritis only marginally. The titers of anti-type II collagen antibodies were not different in these experimental groups. We next evaluated gene expression of inguinal lymph nodes and foot with quantitative PCR. Among cytokines important for arthritis progression, TNF-alpha, IL-17A, and IL-1beta were significantly suppressed. A suppressive cytokine, IL-10, was not upregulated in B47+Foxp3 group. In inguinal lymph nodes, the expression of TNF-alpha, IL-17A, and IL-1beta were not suppressed in B47+Foxp3 group. On histologic examination, although control groups showed severe inflammation, B47+Foxp3 group showed only marginal inflammation. We graded mononuclear cell infiltration and cartilage/bone destruction by the pannus invasion with histopathological examination. Pannus formation was clearly suppressed in the B47+Foxp3 group. These results suggest that regulatory T cells in the arthritis site suppress bone destruction as well as inflammation. In contrast, Foxp3-transduced T cells without antigen specificity were not sufficient for arthritis suppression. Reconstituted regulatory cells also showed effective suppression when transferred after the onset of arthritis when the average arthritic score reached around 2 point. Foxp3-expressing T cells with arthritis-associated TCRs were evidently effective in arthritis suppression. Once activated, regulatory T cells exhibit suppression in an antigen-nonspecific manner.[27] However, antigen specificity is important in migration and expansion of regulatory T cells.[28,29] Indeed, antigen-specific regulatory T cells are efficient in suppression of various autoimmune diseases. The problem is how to obtain enough organ-antigen-specific regulatory T cells for therapeutic transfer. TCR and Foxp3 gene transfer is one possible approach to overcome this problem. In the CIA treatment of polyclonal regulatory T cells, 20 mouse spleens were required to treat 1 mouse.[30] In contrast, about a quarter of splenocytes were used to treat one mouse in our experiment.

CONCLUSION

Taking these results together, the system illustrated in the FIGURE 2 was shown to be feasible for use in experimental animals. Recently, the clinical appreciation of retroviral TCR gene transfer was reported in the treatment of melanoma patients. T cells transduced with melanoma antigen-specific TCRs suppressed disease progression in patients with advanced melanoma.[31] This result showed the essential efficacy and safety of TCR gene transfer in the human. Therefore, autoimmune disease can be a suitable target for TCR gene transfer.

We propose that TCR gene cloning, using the information of TCR clonal analysis and reconstitution of the TCR function by gene transfer, would be a promising strategy for antigen-specific immunotherapy in autoimmune disorders.

ACKNOWLEDGMENTS

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Lessons from a Genomewide Association Study of Rheumatoid Arthritis

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Rheumatoid arthritis is a chronic inflammatory disorder in which the articular joints are gradually destroyed. Occasionally there is systemic involvement, which can include vasculitis in various organs and pulmonary fibrosis. The disease has multifactorial causes to which genetic and environmental factors are thought to contribute. The presence of autoantibodies to cyclic citrullinated peptide (CCP) is specific to rheumatoid arthritis; although the mechanistic significance of these autoantibodies is obscure, their detection contributes both to the differential diagnosis and to a prediction of the severity of joint destruction. Rheumatoid arthritis causes substantial morbidity and mortality and is sometimes accompanied by severe infection or accelerated atherosclerosis.

During the past couple of decades, therapy for rheumatoid arthritis has been improved through the introduction of new antirheumatic drugs, such as the antimetabolite and antifolate drug methotrexate, and biologic therapeutics, such as antagonists to tumor necrosis factor (TNF). However, these treatments can have adverse effects,[1] and responsiveness to these treatments varies considerably. Perhaps "personalized medicine" may one day address such variation. An improved understanding of the genetic causes of the disease represents a step toward this goal and the development of other therapeutic approaches.

The article by Plenge et al.[2] in this issue of the *Journal* is therefore welcome. The authors report the results of a genomewide association study of an anti–CCP-positive subclass of rheumatoid arthritis. It is reassuring that the authors observed associations between rheumatoid arthritis and loci in and around *HLA-DRB1* and *PTPN22*; these loci have been repeatedly implicated as genetic risk factors in persons of European descent. The authors also identified a new locus, containing *TRAF1* and *C5*, through the use of a multistage study design with multiple samples. An earlier large-scale linkage-disequilibrium study and subsequent replication studies implicated a variant of *PADI4* as a risk factor for rheumatoid arthritis.[3] This variant would seem to have a more potent effect in Asian populations than in those of European descent. Variants of these genes are believed to confer a risk for the development of rheumatoid arthritis by affecting the presentation of autoantigens (in the case of *HLA-DRB1*), T-cell–receptor signal transduction (in the case of *PTPN22*), and the citrullination of proteins, targets of anti-CCP antibodies (in the case of *PADI4*). Variant *TRAF1* may modify signal transduction through TNF receptors 1 and 2; variant *C5* may amplify complement activation in the joints of patients with rheumatoid arthritis.

The genomewide association approach has yielded a wealth of new genetic susceptibility loci in other common and complex genetic disorders.[4] The completion of the Human Genome Project and the development of large-scale public databases of human genetic heterogeneity and high-throughput genotyping technology have enabled researchers to carry out case–control association studies on thousands of samples with several hundreds of thousands of markers throughout the human genome. The scale of genomewide association studies continues to grow, and the number of markers used in such studies will soon approximate or exceed a million. It is not unrealistic to expect that the entire genome of all samples will be sequenced in the not-too-distant future.

An advantage of genomewide association studies, as compared with more typical association studies that test for a connection between disease and candidate-gene markers, is that they screen most of the genes in the human genome — thus allowing the investigator to identify new mechanisms of disease susceptibility. Because genomewide association mapping is achieving ever higher resolution and studies are realizing stronger statistical power with every increase in sample size, it is likely that we will have a catalogue of genes with variants for susceptibility to rheumatoid arthritis with various degrees of risk within several years.

What is the principal challenge to the application of findings from genetic mapping studies to clinical medicine? The variant-specific functional differences responsible for common diseases are smaller than those of mutations responsible

for monogenic disorders, so showing that such variants have a relevant biologic effect (in the form of functional data) is often difficult. Most reports of genomewide association studies, including the one by Plenge et al., do not include tests of function of the identified polymorphisms. In seeking to understand the mechanisms of disease and apply this knowledge to improve diagnosis and treatment in the clinical setting, it is necessary to identify the true causal variants and fathom their effect on gene function. More sensitive and sophisticated methods in molecular biology and immunology are required for the investigation of potentially functional variants — the fruits of current and forthcoming genomewide association studies.

Also critical to reaping the full harvest of clinically relevant information from such an approach is the study of populations of various ancestries. Plenge et al. observed that the odds ratios and P values for the identified polymorphisms were weak in the Swedish population. Moreover, the TRAF1–C5 locus did not surface in the Wellcome Trust Case Control Consortium study, which included genomewide association of British persons with rheumatoid arthritis.[4] Although the association of PAD14 and rheumatoid arthritis has been replicated by several large-scale studies in Japanese populations and a Korean population, it has not been observed in the majority of studies of populations of European descent.

One explanation for the differential detection of specific loci is that genetic variants that are associated with rheumatoid arthritis may be enriched in one population and not another or may interact with regional environments and thereby assume influence on disease susceptibility. This hypothesis underscores the need for global collaboration and comparisons of multiple ethnic populations. Personalized medicine in the era of genomewide association studies will make few advances unless the scope of clinical research is broadened to include data from many ethnic groups; non-European populations are currently underrepresented.

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IL-17B and IL-17C Are Associated with TNF-α Production and Contribute to the Exacerbation of Inflammatory Arthritis[1]

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IL-17A is a T cell-derived proinflammatory cytokine that contributes to the pathogenesis of rheumatoid arthritis. Recently, six related molecules have been identified to form the IL-17 family, as follows: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. Whereas IL-17A and IL-17F up-regulate IL-6 in synovial fibroblasts, IL-17B and IL-17C are reported to stimulate the release of TNF-α and IL-1β from the monocytic cell line, THP-1 cell. However, their detailed function remains to be elucidated. We report in this study the effects of IL-17 family on the collagen-induced arthritis (CIA) progression by T cell gene transfer and bone marrow chimeric mice. The mRNA expressions of IL-17 family (IL-17A, IL-17B, IL-17C, and IL-17F) and their receptor (IL-17R and IL-17Rh1) genes in the arthritic paws of CIA mice were elevated compared with controls. Although IL-17A and IL-17F were expressed in CD4$^+$ T cells, IL-17B and IL-17C were expressed in the cartilage and in various cell populations in the CIA arthritic paws, respectively. In vitro, IL-17A, IL-17B, IL-17C, and IL-17F induced TNF-α production in mouse peritoneal exudate cells. In vivo, adoptive transfer of IL-17B- and IL-17C-transduced CD4$^+$ T cells evidently exacerbated arthritis. Bone marrow chimeric mice of IL-17B and IL-17C exhibited elevated serum TNF-α concentration and the high arthritis score upon CIA induction. Moreover, neutralization of IL-17B significantly suppressed the progression of arthritis and bone destruction in CIA mice. Therefore, not only IL-17A, but also IL-17B and IL-17C play an important role in the pathogenesis of inflammatory arthritis. *The Journal of Immunology*, 2007, 179: 7128–7136.

Interleukin-17A is a T cell-derived proinflammatory cytokine that is involved in the development of rheumatoid arthritis (RA).[3] IL-17A was originally named CTLA-8 after being cloned from activated T cells, and shares 57% homology to the protein encoded by the open reading frame 13 gene of the T lymphotropic herpesvirus saimiri (1). IL-17A is present at significant levels in the synovium and synovial fluid of patients with RA (2, 3). IL-17A is a potent inducer of various cytokines such as IL-1, TNF-α, and IL-6. T cell IL-17A stimulates the production of IL-1 and TNF-α from human PBMC-derived macrophages in vitro (4). IL-17A also enhances IL-1-mediated IL-6 production by RA synoviocytes in vitro as well as TNF-α-induced synthesis of IL-1, IL-6, and IL-8 (5, 6). These results indicate that IL-17A synergizes with IL-1 and TNF-α and contributes to inflammation of RA.

In in vivo studies, systemic as well as local overexpression of IL-17A in collagen-induced arthritis (CIA) has been shown to accelerate the onset of CIA and to aggravate the joint pathology (7). Moreover, treatment with anti-IL-17A Abs after the onset of CIA reduces the joint inflammation and histologic destruction of cartilage (8). IL-17A deficiency protects IL-1R antagonist-deficient mice from spontaneous development of destructive arthritis (9). Therefore, IL-17A plays a crucial role in the pathogenesis of arthritis through synergistic effects with IL-1 and TNF-α. However, IL-17A can directly induce joint destruction in an IL-1-independent manner and can bypass TNF-dependent arthritis (7, 10). This suggests that there is an IL-17A-dependent pathway to the destructive arthritis and anti-IL-17A cytokine therapy is an additional new antirheumatic strategy for RA besides anti-TNF/anti-IL-1 therapy.

Recently, the IL-17 family was determined to consist of six related molecules, as follows: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. These molecules have a molecular mass of 20–30 kDa and consist of 163–202 aa that bear 20–50% homology to IL-17A, especially within the C-terminal region. They share four conserved cysteine residues that may participate in the formation of intermolecular disulfide linkages (11, 12). The different IL-17 family members seem to have very distinct expression patterns, suggesting distinct biological roles.

Interestingly, IL-17F has the highest homology with IL-17A and is also expressed by activated T cells in response to IL-23 stimulation (13–15). However, the precise effect of IL-17F on arthritis has not been clarified. In contrast to the restricted expression of IL-17A and IL-17F, IL-17B mRNA can be detected in a wide range of tissues, including the spinal cord, testis, stomach, small intestine, pancreas, prostate, and ovary (16, 17). It has been recently reported that IL-17B is highly expressed in chondrocytes

that are located at the mid and deep zones of normal bovine articular cartilage (11). In contrast, IL-17C expression has been confined only to rare expression sequence tags in adult prostate and fetal kidney libraries (17). However, the cell sources of IL-17B and IL-17C have not been identified in the development of inflammatory arthritis.

A common feature of IL-17 family members is the induction of neutrophil migration. IL-17A and IL-17F both mobilize neutrophils partly through granulopoiesis and CXC chemokine induction (12). Intranasal administration of adenovirus expressing IL-17A, IL-17C, or IL-17F resulted in neutrophilia in the bronchoalveolar lavage (18). Moreover, i.p. injection of human rIL-17B caused marked neutrophil migration in normal mice (17). In contrast, the members can be divided into two groups according to the induction of cytokine production. Although IL-17A and IL-17F up-regulate IL-6 and IL-8 in human fibroblasts (19, 20), IL-17B and IL-17C are reported to stimulate the release of TNF-α and IL-1β from the monocytic cell line THP-1 (17). Taken together, these results indicate that IL-17 family members induce inflammatory cytokines not only through activated T cells, but also through activated monocytes/macrophages.

Based on the structural and functional similarities among IL-17 family members, we speculated that not only IL-17A, but also other IL-17 family members are involved in the pathogenesis of many inflammatory and autoimmune disorders, especially in the development of RA. We focused on IL-17A, IL-17B, IL-17C, and IL-17F, which can affect inflammatory cytokine production of fibroblasts and macrophages. Recently, IL-17C expression in synovial fluid mononuclear cells and PBMCs of RA patients was reported (21). However, the biological effect of IL-17 family members in arthritis has not been analyzed.

In the present study, we investigated the expression and effect of IL-17 family members in arthritis. In vitro, not only IL-17A, but also IL-17B and IL-17C induced the mRNA expression of inflammatory cytokines such as IL-1β, IL-6, and IL-23 in the 3T3 cell line and peritoneal exudate cells (PECs). The supernatant of the PECs stimulated with each IL-17 family member all increased TNF-α production significantly compared with controls. In vivo, CD4$^+$ T cells transduced with each of IL-17B, IL-17C, or IL-17F exacerbated CIA in mice to the same degree as CD4$^+$ T cells transduced with IL-17A. Mice reconstituted with bone marrow (BM) cells transduced with each of IL-17B, IL-17C, or IL-17F suffered from severe CIA. Moreover, neutralization of IL-17B significantly suppressed the progression of arthritis and bone destruction in CIA mice. Our results suggest that not only IL-17A, but also the other IL-17 family members (IL-17B, IL-17C, and IL-17F) are associated with inflammatory cytokines such as IL-1 and TNF-α and contribute to the exacerbation of autoimmune arthritis.

Materials and Methods

Animals

DBA/1J mice were purchased from Japan SLC. All mice were used at 6–8 wk of age. All animal experiments were conducted in accordance with the institutional and national guidelines.

Collagen-induced arthritis

CIA was induced, as described previously (22–24). In brief, bovine type II collagen (BCII) (Chondrex) was emulsified with an equal volume of CFA (Chondrex). DBA/1J mice were immunized intradermally at the base of the tail with 100 μg of BCII emulsified with CFA. On day 21, the mice were boosted by intradermal injection with 100 μg of BCII emulsified with IFA (Difco). The arthritis score was determined by erythema, swelling, or ankylosis per paw, as described previously (25, 26). The clinical arthritis score was defined as the sum of the scores of all four paws of each mouse.

Cytokines and cell lines

Recombinant murine IL (mIL)-17A, mIL-17B, mIL-17C, and mIL-17F were obtained from R&D Systems. The mouse fibroblast cell line 3T3 was obtained from American Type Culture Collection. This cell line was cultured with RPMI 1640 (Invitrogen Life Technologies) medium supplemented with 10% FCS, 2 mM γ-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 × 10^{-5} M 2-ME. Ba/F3 cells were maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM γ-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 ng/ml rmIL-3 (R&D Systems).

Murine PECs

Murine PECs were isolated after i.p. injection of 3 ml of 5% sterile fluid Brewer's thioglycolate broth (Sigma-Aldrich) into 8-wk-old DBA/1J mice (27). After culture of the PECs in a 6-well plate for 2 h, floating cells were removed by extensive washing, and attached cells were maintained in the medium described above for 3 days. More than 80% of the cultured cells were macrophages as determined by flow cytometric analysis of CD11b-positive cells. The following recombinant murine cytokines were added to the culture medium and incubated for 24 h: 50 ng/ml mIL-17A, mIL-17B, mIL-17C, or mIL-17F.

Preparation of retroviral constructs of mIL-17 family cDNAs

mIL-17A, mIL-17B, mIL-17C, and mIL-17F were isolated from the murine T lymphocyte cDNA library according to the reported nucleotide sequence from National Center for Biotechnology Information (mIL-17A NM_010552; mIL-17B NM_019508; mIL-17C NM_145834; mIL-17F NM_145856). The full-length fragments were subcloned into retrovirus vector murine stem cell virus/internal ribosome entry site/GFP (pMIG), as described previously (28).

Production of retroviral supernatants and retroviral transduction

Retroviral supernatants were obtained by transfection of pMIG carrying each of the IL-17 family genes into PLAT-E packaging cell lines using FuGENE 6 transfection reagent (Roche Diagnostic System), as described previously (29). For the detection of GFP-positive cells, we used an EPICS XL flow cytometer (Beckman Coulter).

Gene transduction to mouse splenocytes and adoptive transfer

Total splenocytes were cultured for 48 h in the presence of Con A (10 μg/ml) (Sigma-Aldrich) and mIL-2 (50 ng/ml) (R&D Systems). Retroviral gene transduction was performed, as described previously (30, 31). A CD4$^+$ T cell population was prepared by negative selection by MACS with anti-CD19 mAb, anti-CD11c mAb, and anti-CD8a mAb (BD Pharmingen). The gene-transduced CD4$^+$ T cells were suspended in PBS and injected i.v. (1 × 10^7) at 23 days after the first immunization of BCII.

BM precursor cell isolation, infection, and transfer

BM precursor cell isolation, retrovirus infection, and transfer were performed, as described previously (32). In brief, DBA/1J mice were treated with 5 mg/body 5-fluorouracil (Sigma-Aldrich) dissolved in PBS. After 5 days, BM cells were harvested and cultured with 50 ng/ml mIL-3, mIL-6, and murine stem cell factor (R&D Systems) for 48 h. Then the BM cells were spin infected with the retrovirus supernatants with 16 μg/ml polybrene (Sigma-Aldrich) for 90 min at 2400 rpm and 25°C. Recipient mice were treated by 700 rad of whole-body radiation and were injected with 1 × 10^6 of the BM cells i.v. Recipient mice were maintained for 6 wk until analysis or immunization.

RNA isolation, cDNA synthesis, and quantitative real-time PCR

RNA of the cells was extracted using an RNeasy Micro Kit and RNeasy Mini Kit (Qiagen). RNA from the tissues was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method using ISOGEN (Nippon Gene). RNA was reverse transcribed to cDNA with random primers (Invitrogen Life Technologies) and Superscript III, according to the manufacturer's protocol (Invitrogen Life Technologies). To determine the cellular expression of each protein, quantitative real-time PCR analysis was performed using an iCycler (Bio-Rad). The PCR mixture consisted of 25 μl of SYBR Green Master Mix (Qiagen), 15 pmol of forward and reverse primers, and the cDNA samples, in a total volume of 50 μl. We calculated the quantitative PCR data with δ cycle threshold method, and relative RNA abundance was determined based on control β-actin abundance. To measure the relative efficiency,

amplifications were performed on the serial diluted cDNA samples using primers for the target and the reference (β-actin) genes. We made plots of the log cDNA dilution vs δ cycle threshold, and confirmed that the efficiencies of the target and the reference genes were similar because the absolute value of the slope was close to zero (data not shown) (33, 34). The primer pairs used in the quantitative real-time PCR were as follows: mouse IL-17A, sense 5'-GCTCCAGAAGGCCCTCAGA-3', antisense 5'-AGCTTTCCCTCCGCATTGA-3'; mouse IL-17B, sense 5'-CGGTGCCTATGTTTGGGTTGC-3', antisense 5'-GGGTTG GTGGTTGGCTCAGAA-3'; mouse IL-17C, sense 5'-CACAGATGAG AACCGCTACCC-3', antisense 5'-GCGGATGAACTCGGTGTGGA A-3'; mouse IL-17F, sense 5'-CAACGCTGCATACAAAAATCA-3', antisense 5'-TTAAGTGAGGCATTGGGAACA-3'; mouse IL-17R, sense 5'-CCACTCTGTAGCACCCCAATG-3', antisense 5'-CCTGGA GATGTAGCCCTGGTC-3'; mouse IL-17Rh1, sense 5'-GCAAGGAA GGAGCACGAAGAC-3', antisense 5'-CTCGGCGATTTTCTTTTTCT G-3'; mouse TNF-α, sense 5'-CATCTTCTCAAAATTCGAGTGACA A-3', antisense 5'-TGGGAGTAGACAAGGTACAACCC-3'; mouse IL-1β, sense 5'-CAACCAACAAGTGATATTCTCCATG-3', antisense 5'-GATCCACACTCTCCAGCTGCA-3'; mouse IL-6, sense 5'-CACT TCACAAGTCGGAGGCTTA-3', antisense 5'-GCAAGTGCATCATC GTTGTTC-3'; mouse IL-23, sense 5'-TGGCATCGAGAAACTGTGAG A-3', antisense 5'-TCAGTTCGTATTGGTAGTCCTGTTA-3'; and mouse β-actin, sense AGAGGGAAATCGTGCGTGAC-3', antisense 5'-CAATAGTGATGACCTGGCCGT-3'.

Immunoassays of cytokines and anti-type II collagen Ab

The concentrations of mIL-6, mTNF-α, and mIL-17A in mouse sera and culture supernatants were measured by sandwich ELISA, according to the manufacturer's protocol (BD Pharmingen). An automatic microplate reader (Bio-Rad 550) was used to measure the OD. Mouse serum IgG anti-type II collagen Ab titer was measured, as previously described (35).

Isolation of cartilage

Murine articular cartilage was isolated from patellae, as described previously (36). In brief, patellae were decalcified in 3.5% EDTA for 4 h at 4°C, when the whole cartilage layer was stripped off. Because old cartilage is more calcified, decalcification of the patellae of old mice (>3 mo) was performed overnight at 4°C.

Cell purification

Briefly, the arthritic paws of the CIA mice were cut into pieces, digested with collagenase type IV (Sigma-Aldrich), and stained with mAbs (Fc blocking with anti-mouse CD16/CD32 mAb, and staining with anti-mouse CD3-PE mAb, anti-mouse CD4-allophycocyanin mAb, anti-mouse CD11b-FITC mAb, anti-mouse CD11c-FITC mAb, anti-mouse CD19-FITC mAb, biotinylated anti-mouse I-A/I-E (MHC class II) mAb, and streptavidin PE Ab that were obtained from BD Pharmingen). Cell sorting of a specific cell population was performed with a FACSVantage flow cytometer (BD Biosciences).

Intracellular cytokine staining and flow cytometry

IL-17 family expressions of Ba/F3 cells transduced with each of IL-17 family members were examined using intracellular cytokine staining. Ba/F3 cells were infected with the retroviral supernatants in the presence of 10 μg/ml polybrene (Sigma-Aldrich) for 120 min. These cells were stained with anti-mouse IL-17A mAb conjugated to PE (BD Pharmingen), biotinylated anti-mouse IL-17B polyclonal Ab (R&D Systems), anti-mouse IL-17C polyclonal Ab (R&D Systems), and anti-mouse IL-17F mAb (R&D Systems), respectively. Bovine anti-goat IgG-PE (Santa Cruz Biotechnology) and F(ab')$_2$ goat anti-rat IgG PE (Serotec) were used as secondary reagents for IL-17C and IL-17F staining, respectively. Cell fixation and permeabilization were performed using Cytofix/Cytoperm reagent (BD Pharmingen), according to the manufacturer's protocol (BD Pharmingen), and analyzed by flow cytometry. Splenocytes isolated from BM chimeric mice of IL-17A were also stained with anti-mouse IL-17A mAb in the same way.

Anti-IL-17B Ab treatment in CIA mice

CIA was induced in DBA/1J mice, as described above. Mice exhibited the first clinical signs of arthritis (arthritis score between 1 and 2) and were injected i.p. with 100 μg of polyclonal anti-mouse IL-17B Abs (R&D Systems). PBS was i.p. injected as a control. Arthritis was assessed using a scoring system, as described above. Mice were sacrificed at 10 days after the onset of arthritis, and the paws were removed. Joint pathology was evaluated on decalcified H&E-stained sections.

Histopathology

The tarsal joints of sacrificed CIA mice were embedded in paraffin wax after 10% formaldehyde fixation and decalcification. The sections were stained with H&E. Synovial tissues were graded by mononuclear cell infiltration and pannus invasion, as described previously (37).

Statistical analysis

Data are expressed as the means ± SD. All results were obtained by at least three independent experiments. Statistical significance was determined by the Mann-Whitney U test and unpaired Student's t tests. A value of $p <$ 0.05 was considered statistically significant.

Results

IL-17 family genes (IL-17A, IL-17B, IL-17C, and IL-17F) were highly expressed in the arthritic paws of CIA mice

First, we examined the expressions of IL-17 family members and IL-17Rs in the arthritic paws of CIA mice by quantitative PCR. The mRNA expressions of all IL-17 family genes examined (IL-17A, IL-17B, IL-17C, and IL-17F) were highly elevated in the arthritic paws compared with the controls. In accordance with previous report of high in vivo expression of IL-17R in RA (38), mRNA expressions of IL-17Rs (IL-17R and IL-17Rh1) were also elevated (Fig. 1A). As expected, the mRNA expressions of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-23) were also elevated in the arthritic paws compared with controls (Fig. 1B).

We next examined cell populations in the arthritic paws of CIA mice that express IL-17 family members. Subpopulations of the cells were sorted with various cell surface markers using a flow cytometer. As expected, CD4$^+$ T cells expressed IL-17A and IL-17F significantly. IL-17B was expressed exclusively in the inflammatory cartilage of CIA mice. In contrast, IL-17C was expressed in a broad range of cells, i.e., CD4$^+$ T cells. CD11b$^+$ MHC class II$^+$ macrophages, and CD11c$^+$ MHC class II$^+$ dendritic cells (Fig. 1C). These results suggested that CD4$^+$ T cells mainly express IL-17 family members, especially IL-17A, IL-17C, and IL-17F, at the inflammatory site.

IL-17 family induced several proinflammatory cytokines

We next investigated whether IL-17 family members have an influence on mouse fibroblast cell lines and mouse peritoneal macrophages. Cells of the mouse fibroblast line 3T3 were cultured with each of the IL-17 family members (50 ng/ml), and cytokine expression was examined after 24 h of incubation. IL-17A induced IL-1β and IL-6 expressions, as previously reported (2). Moreover, IL-17B, IL-17C, and IL-17F also induced IL-1β expression in 3T3 (Fig. 2A).

To examine the effects of IL-17 family members on mouse macrophages, thioglycolate-elicited PECs were isolated and cultured with each of the IL-17 family members (50 ng/ml). IL-17A induced IL-1β, IL-6, and IL-23 expressions in PECs. Interestingly, IL-17B also induced IL-1β, IL-6, and IL-23 expressions. Moreover, IL-17C induced IL-1β and IL-23 expressions in PECs (Fig. 2B). In addition, PECs stimulated with every IL-17 family member produced significantly increased amount of TNF-α protein compared with the control, and PECs stimulated with IL-17A and IL-17B produced significantly increased amount of IL-6 protein (Fig. 2C). These results suggested that IL-17A. IL-17B, IL-17C, and IL-17F stimulate fibroblasts and macrophages to produce inflammatory cytokines.

FIGURE 1. The expression of IL-17 family members and IL-17R genes in the arthritic paws of CIA mice. A, The expressions of IL-17 family genes and IL-17R genes were examined in the arthritic paws of CIA mice (■; n = 3) and in control mice (□; n = 3) by quantitative PCR. B, The expressions of inflammatory cytokines. C, The expressions of IL-17 family members in the sorted cell populations of the arthritic paws of CIA mice. The data are representative of three independent experiments.

Exacerbation of CIA by transfer of IL-17 family-transduced CD4⁺ T cells
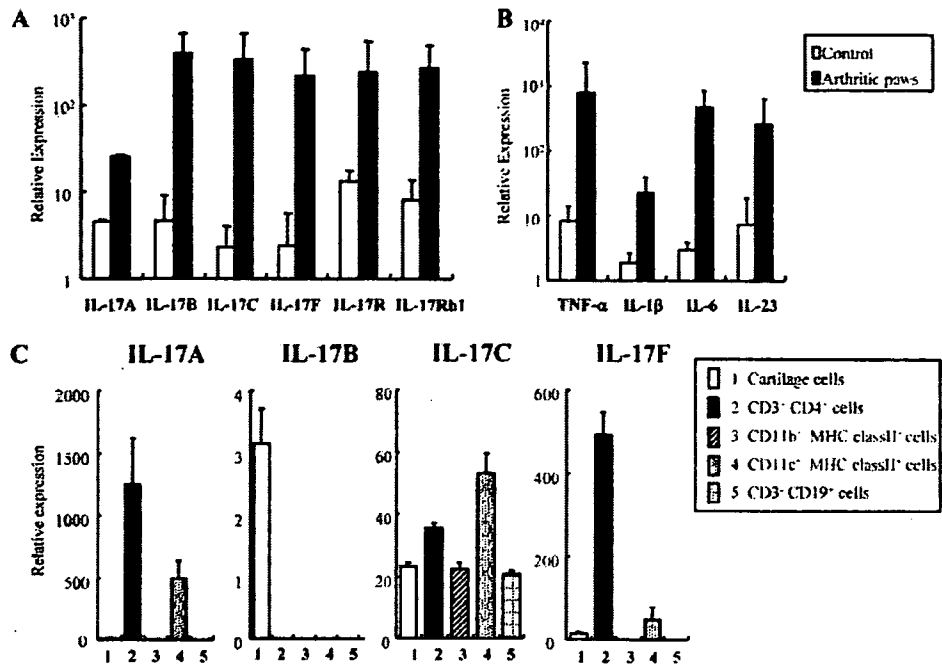
Exacerbation of CIA by transfer of IL-17 family-transduced CD4⁺ T cells

Because IL-17B and IL-17C induce the expression of inflammatory cytokines in fibroblasts and macrophages, we hypothesized that IL-17B and IL-17C have an effect on the process of arthritis. We subcloned cDNA fragment of mIL-17A, mIL-17B, mIL-17C, or mIL-17F to pMIG retrovirus vector. These vectors were retrovirally transduced to Ba/F3 cells, and protein expressions of IL-17 family members were confirmed with intracellular staining of each IL-17 family cytokine (Fig. 3A).

To examine the proinflammatory effects of the IL-17 family in vivo, we retrovirally transduced the IL-17 family genes to CD4⁺ T cells. The transduction efficiencies were ~30% on average (Fig. 3B). These IL-17 family-transduced CD4⁺ T cells were adoptively transferred to BCII-immunized DBA1 mice before the onset of arthritis. They exacerbated the progression of arthritis, as observed by the arthritis score (Fig. 3, C and D). The IL-17 family member-transduced CD4⁺ T cells had no significant effect on the serum levels of anti-BCII IgG Abs at 14 days after the onset of CIA (data not shown). These results
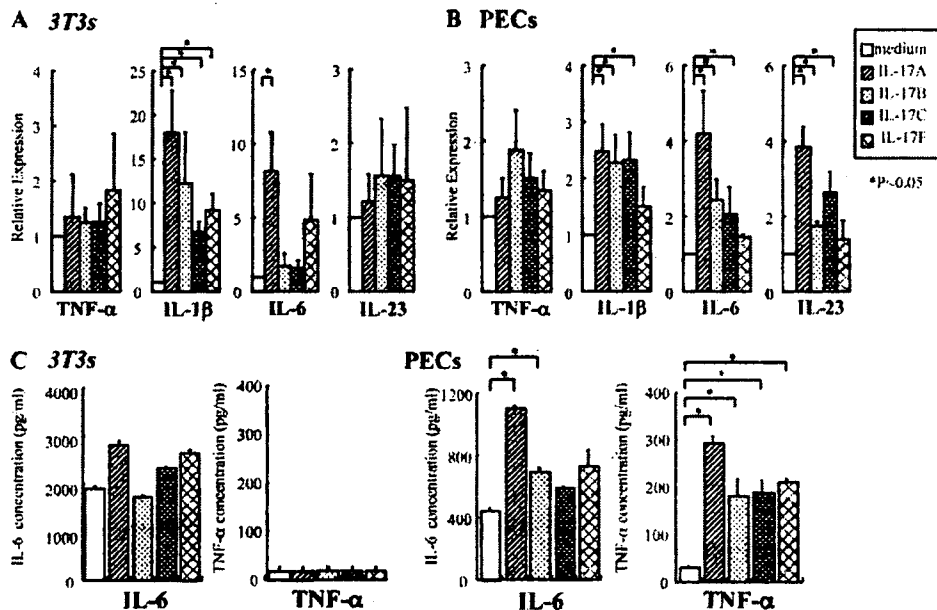
FIGURE 2. The proinflammatory effects of IL-17 family members on mouse fibroblasts and macrophages. A, Relative expression of the cytokine genes in 3T3 cell. The mouse fibroblast cell line 3T3 was cultured with each of mIL-17A, mIL-17B, mIL-17C, or mIL-17F for 24 h, and the expressions of inflammatory cytokines were measured by quantitative PCR. B, Relative expression of the cytokine genes in mouse thioglycolate-elicited PECs. PECs were cultured with each of mIL-17A, mIL-17B, mIL-17C, or mIL-17F for 24 h, and the expressions of inflammatory cytokines were measured by quantitative PCR. C, The secreted IL-6 and TNF-α levels in the supernatants of 3T3 and PECs were measured by ELISA. Error bars indicate ± SD. The data are representative of three independent experiments. Significance of differences between control (medium) and each IL-17 family was determined: *, p < 0.05.

FIGURE 3. The effects of transfer of IL-17 family-transduced CD4^{+} T cells on CIA. *A*, Intracellular IL-17 family expressions in Ba/F3 cells retrovirally transduced with each IL-17 family member. GFP-gated IL-17 family-transduced (mIL-17A, mIL-17B, mIL-17C, or mIL-17F) Ba/F3 cells were analyzed for IL-17A, IL-17B, IL-17C, or IL-17F expression compared with GFP-gated empty vector (pMIG)-transduced Ba/F3 cells. *B*, Representative FACS analysis of IL-17 family-transduced CD4^{+} T cells was shown. Numbers in dot plots indicate the percentage of GFP^{+} CD4^{+} and GFP^{-} CD4^{+} cells, and the percentages of the GFP^{+} cells within total CD4^{+} cells were shown below. *C* and *D*, CD4^{+} T cells transduced with each of IL-17 family genes were transferred to collagen-immunized mice before the onset of arthritis (day 23). The incidence of arthritis (*C*) and the progression of arthritis scores (*D*) are shown. Values are the mean of arthritis score (*n* = 20 mice per group). Significance of differences between control (pMIG) and each IL-17 family-transduced mice was determined; **, $p < 0.005$; *, $p < 0.05$.
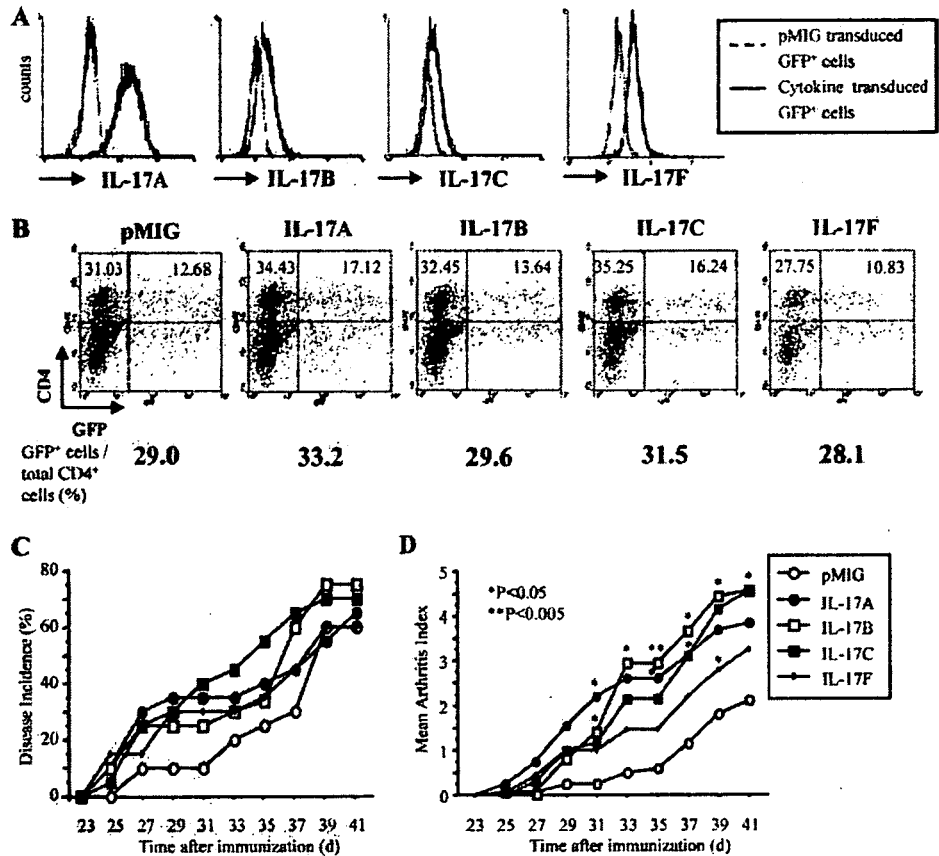
indicated that the effect of IL-17 family members on the progression of arthritis was not associated with the elevations of anti-BCII Abs.

IL-17 family BM chimeric mice exhibited high arthritis scores upon CIA induction

To examine the proinflammatory effect of constitutively expressed IL-17 family members, we established IL-17 family BM chimeric mice by transfer of gene-transduced BM cells to lethally irradiated mice. In a previous study, the attempt to generate IL-17A-overexpressing mice with a conventional transgenic approach was unsuccessful because these mice were embryonic lethal (39). In accordance with the previous report, mice that expressed IL-17A with high efficiency (i.e., for which the percentage of GFP^{+} cells in the spleen was >50%) became gaunt and died within 1 mo after BM transplantation (data not shown). When the percentage of GFP^{+} cells in the spleen was 5–15%, the mice appeared to be healthy for several months. We therefore used BM chimeric mice that expressed IL-17 family genes in ~5–15% of spleen cells. Eight weeks after the BM transplantation, mIL-17A was readily detected by intracellular cytokine staining (Fig. 4*A*). Moreover, the serum concentration of mIL-17A was significantly elevated in these mice (Fig. 4*B*). Therefore, the BM chimeric mice were successfully allowed to express the transduced cytokines systemically. Then we immunized these mice with BCII 8 wk after BM transplantation. BM chimeric mice of IL-17A and IL-17F exhibited early onset and high arthritis scores upon CIA induction (Fig. 5, *A* and *B*). BM chimeric mice of IL-17B and IL-17C clearly exacerbated arthritis, as assessed by the arthritis score. In contrast, BM chimeric mice of IL-17B and IL-17C did not result in significant differences in the onset of disease (Fig. 5, *C* and *D*). BM ex-

pression of IL-17 family member did not affect the anti-BCII Ab responses at 14 days after the onset of CIA (data not shown). These results indicated that the effect of IL-17 family members on the exacerbation of arthritis was not associated with the responses of anti-BCII Abs.
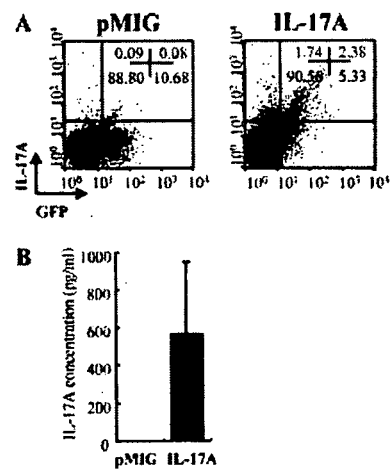
FIGURE 4. Generation of IL-17 family chimeric mice by BM transplantation of gene-transduced BM cells. Each of IL-17 family genes was transduced to BM cells with retrovirus vector and transferred to lethally irradiated mice. *A*, The intracellular expression of IL-17A protein in the spleen of IL-17A BM chimeric mice 8 wk after BM transplantation. The percentage of GFP^{+} cells expressing IL-17A is indicated. The data are representative of three independent experiments. *B*, The concentration of IL-17A protein in the serum of IL-17A BM chimeric mice (*n* = 6) and control mice (pMIG BM chimeric mice) (*n* = 6). The levels of IL-17A were measured by ELISA.

FIGURE 5. Incidence of CIA and arthritis scores in IL-17 family BM chimeric mice. Incidence of CIA and arthritis scores in IL-17A and IL-17F BM chimeric mice (*A* and *B*), and in IL-17B and IL-17C BM chimeric mice (*C* and *D*). Mice were immunized with BCII 8 wk after the BM transplantation. Values are the mean of experiments for IL-17A and IL-17F BM chimeric mice (*n* = 20 per group) and experiments for IL-17B and IL-17C BM chimeric mice (*n* = 30 per group). Significance of differences between control (pMIG) and each IL-17 family BM chimeric mice was determined; $**, p < 0.005$; $*, p < 0.05$. *E*, The mRNA expression of inflammatory cytokines in the spleen of BM chimeric mice of IL-17B and IL-17C, which were immunized with BCII (■; *n* = 15 per group) or nonimmunized controls (□; *n* = 6 per group). Significance of differences between control (pMIG) and each IL-17 family BM chimeric mice was determined; $**, p < 0.005$; $*, p < 0.05$. *F*, The secreted TNF-α and IL-6 levels in the serum of IL-17 family BM chimeric mice that were immunized with BCII (■; *n* = 15) or nonimmunized controls (□; *n* = 6). Significance of differences between control (pMIG) and each IL-17 family BM chimeric mice was determined; $**, p < 0.005$; $*, p < 0.05$.
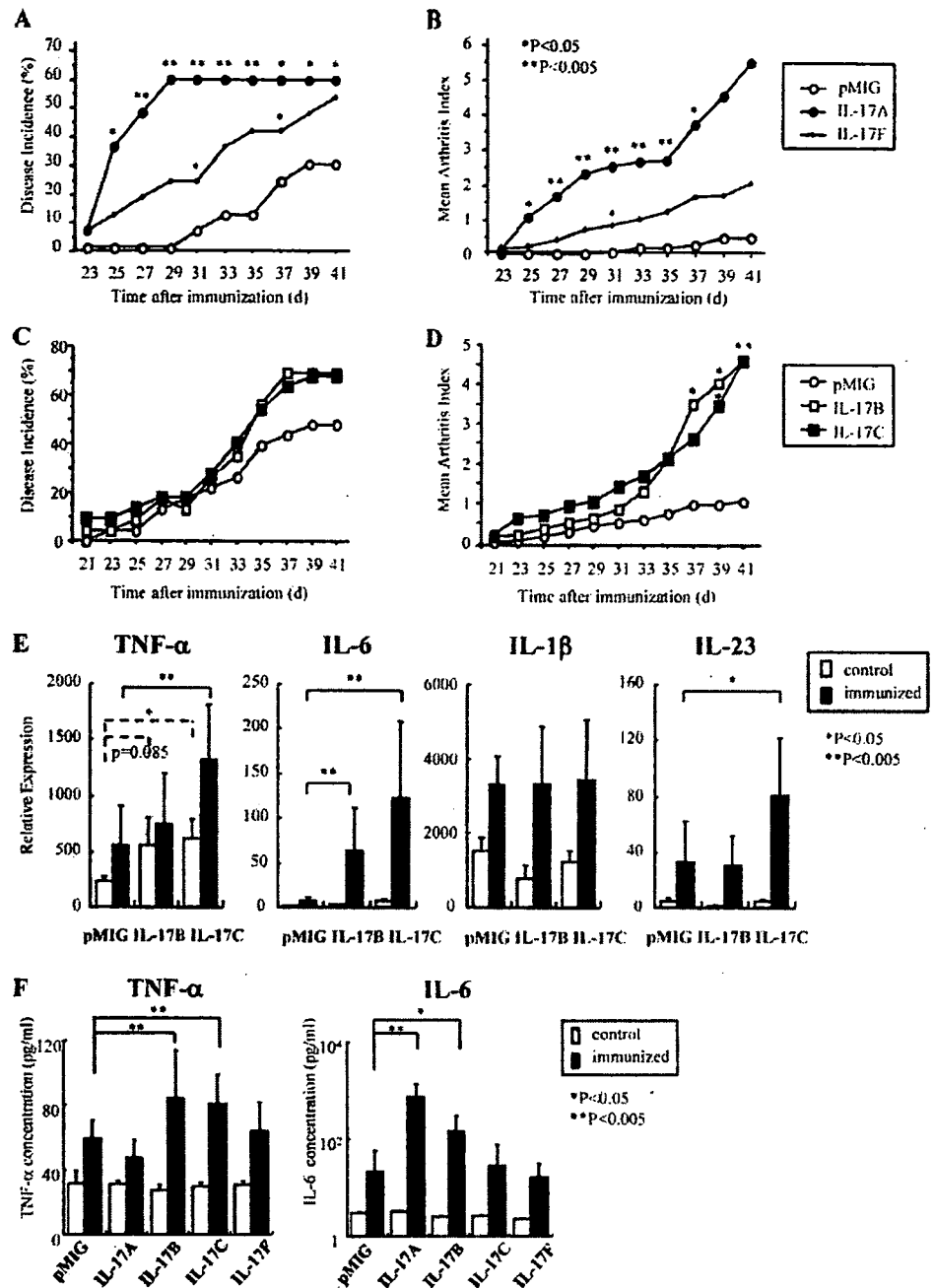
We next examined the alterations of inflammatory cytokine production in these BM chimeric mice. Interestingly, nonimmunized IL-17C BM chimeric mice showed increased mRNA expression of TNF-α in the spleen compared with controls (Fig. 5*E*). Moreover, in the spleen of BCII-immunized IL-17C BM chimeric mice, the mRNA expressions of TNF-α, IL-6, and IL-23 were up-regulated. In contrast, BCII-immunized IL-17B BM chimeric mice showed increased mRNA expression of IL-6 in the spleen compared with controls (Fig. 5*E*). When we examined the concentrations of TNF-α and IL-6 protein in the sera of IL-17 family BM chimeric mice, the BCII-immunized IL-17B and IL-17C BM chimeric mice showed increased TNF-α concentration in the sera. And the BCII-immunized IL-17A and IL-17B BM chimeric mice showed increased IL-6 production in the sera (Fig. 5*F*). These results suggested that IL-

17B and IL-17C enhanced inflammation in this mouse model of arthritis by increased inflammatory cytokine production.

Neutralization of IL-17B significantly suppressed the progression of arthritis

As shown in Fig. 5, we found that IL-17B exacerbated the progression of CIA as well as IL-17A with the method of retrovirus-mediated BM chimeric mice. Regarding IL-17A, neutralizing Abs against IL-17A have been previously shown to be effective in the treatment of CIA (8). We examined the effect of IL-17B blockade in CIA mice. CIA mice were systemically treated with polyclonal anti-mouse IL-17B Abs immediately after the first signs of arthritis. Neutralization of IL-17B significantly suppressed the progression of CIA compared with the controls (Fig. 6*A*). Moreover, histological analysis revealed significant reduction of cell infiltration
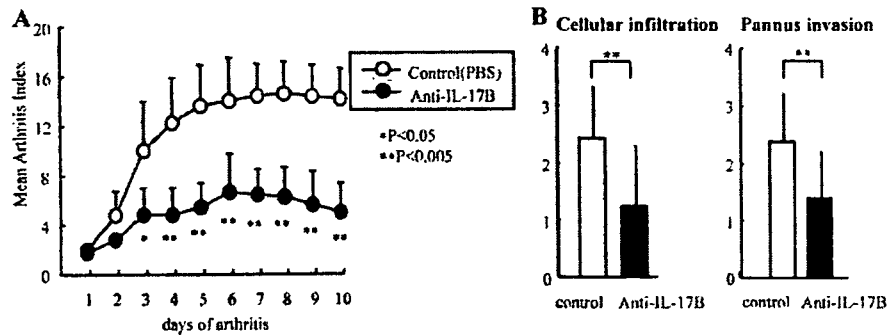
FIGURE 6. Effect of anti-IL-17B Ab treatment in CIA mice. *A*, CIA mice received i.p. injection of anti-mouse IL-17B Abs after the first clinical signs of arthritis (arthritis score between 1 and 2). As a control, PBS was injected. The arthritis score was shown. *B*, Histological score of the inflammatory joints of CIA mice treated with anti-IL-17B Abs was evaluated at 10 days after the onset of arthritis. Cellular infiltration and pannus invasion were graded in all four paws of the mice. Values are the mean of arthritis scores for anti-IL-17B Ab-treated mice and control mice ($n = 5$ per group). Significance of differences between control and anti-IL-17B Ab-treated mice was shown.

and pannus invasion in the anti-IL-17B Ab-treated mice (Fig. 6*B*). These results indicated that IL-17B was associated with the progression of arthritis in CIA mice.

Discussion

RA is considered to be an autoimmune disease, and is characterized by sustained inflammation of the joints and destruction of cartilage and bone. Several inflammatory cytokines are known to mediate the pathogenesis of arthritis, and TNF-α and IL-6 are the most important cytokines in the pathogenesis of RA. IL-17A, IL-17B, IL-17C, and IL-17F have the capacity to induce TNF-α production in PECs in vitro. In vivo, the mRNA expression of TNF-α was spontaneously increased in the spleen of IL-17C BM chimeric mice. Moreover, TNF-α productions in the sera of BCII-immunized IL-17B and IL-17C BM chimeric mice were up-regulated. Although IL-17A induced TNF-α production in PECs, IL-17A BM chimeric mice did not show up-regulated production of TNF-α. This result is consistent with previous observation in THP-1 cell line that IL-17B and IL-17C stimulated the release of TNF-α, whereas IL-17A has only a weak effect on TNF-α (17). In contrast to IL-17B and IL-17C, IL-17A may not be directly associated with TNF-α production in vivo. Moreover, the mRNA expression in the spleen and serum concentration of IL-6 were significantly up-regulated in IL-17B BM chimeric mice that were immunized with BCII. These results showed the close association of IL-17B and IL-17C with TNF-α and IL-6 in vivo and clearly suggested the importance of IL-17B and IL-17C in the pathogenesis of RA.

To date, the cell sources of IL-17B and IL-17C have not been identified. In this study, we showed that IL-17B was expressed in the inflammatory cartilage of CIA mice, whereas IL-17C was expressed in a broad range of cells, i.e., CD4$^+$ T cells, CD11b$^+$ MHC class II$^+$ macrophages, and CD11c$^+$ MHC class II$^+$ dendritic cells. IL-17A and IL-17F were expressed in CD4$^+$ T cells, as expected. These results suggested that CD4$^+$ T cells are involved in the expression of IL-17 family members, especially IL-17A, IL-17C, and IL-17F, at the inflammatory site. Although we did not detect a unique cell source of IL-17C, the arthritis-promoting effect of IL-17C-transduced CD4$^+$ T cells suggests the importance of IL-17C expressed in CD4$^+$ T cells.

In our in vivo analysis, we observed arthritis-promoting effects of the IL-17 family members. As shown in Fig. 3, the transfer of mIL-17A-, mIL-17B-, mIL-17C-, and mIL-17F-transduced CD4$^+$ T cells evidently exacerbated arthritis as assessed by the arthritis score. This effect was also confirmed in the CIA of the mIL-17A, mIL-17B, mIL-17C, and mIL-17F BM chimeric mice. The arthri-

tis-promoting effect of IL-17A was previously reported in a study using adenovirus vector (5, 40). In contrast to IL-17A, which hastened the onset of arthritis, IL-17B and IL-17C did not affect the onset of arthritis evidently. This fact suggests that IL-17B and IL-17C affect arthritis rather in the effector phase. To our knowledge, this is the first observation of an in vivo arthritis-promoting effect of IL-17B and IL-17C.

Blockade of IL-17A has recently been shown to be effective in the treatment of CIA (8). In the present study, we have demonstrated the therapeutic potential of IL-17B blockade after the onset of CIA. Because blockade of TNF-α or IL-1β is not always effective in RA patients, blockade of additional cytokine might be a useful therapeutic option. Therefore, our data strongly suggest that IL-17B as well as IL-17A could be an important target for the treatment of inflammatory arthritis.

In a recent study, the combination of IL-6 and TGF-β was reported to strongly induce IL-17A production in Th17 cells (41). Moreover, it was recently recognized that IL-23 contributes to the expansion of autoreactive IL-17A-producing T cells and promotes chronic inflammation dominated by IL-17A, IL-6, IL-8, and TNF-α (14, 42). Thus, IL-17B and IL-17C may exacerbate arthritis via IL-6- and IL-23-mediated promotion of IL-17A production. However, the possibility that IL-17B and IL-17C exert a cooperative proinflammatory response together with IL-17A and IL-17F in arthritis by regulating the release of cytokines such as IL-6, IL-1β, and IL-23 still remains to be examined.

IL-17F has the highest homology with IL-17A and, like IL-17A, is produced by activated T cells. IL-17F appears to have an effect similar to that of IL-17A on cartilage proteoglycan release and inhibition of new cartilage matrix synthesis (11). Although IL-17F is thought to contribute to the pathology of inflammatory disorders such as RA, the in vivo effect of IL-17F on arthritis was not elucidated. In this study, we found that transduction of BM-expressed IL-17F resulted in both an earlier onset and a subsequent aggravation of arthritis.

We also found that the mRNA expression of all IL-17 family and IL-17R genes examined (mIL-17A, mIL-17B, mIL-17C, mIL-17F, mIL-17R, and mIL-17Rh1) was elevated in the arthritic paws of CIA mice compared with the paws of the control mice. The receptor for IL-17A is IL-17R (also named IL-17AR), which is extensively expressed in various tissues or cells tested, in contrast to the exclusive expression of IL-17A in activated T cells. Recently, IL-17R signaling has been suggested to play a crucial role in driving the synovial expression of proinflammatory and catabolic mediators, such as IL-1, IL-6, matrix metalloproteinase

(MMP)-3, MMP-9, and MMP-13, in streptococcal cell wall-induced arthritis (43). IL-17R-deficient (IL-17R$^{-/-}$) mice that were locally injected five times with streptococcal cell wall fragments into the knee joints showed a significant reduction of joint thickness and cartilage damage that was accompanied by reduced synovial expression of IL-1, IL-6, and the MMPs 3, 9, and 13 compared with arthritic wild-type mice. Therefore, these results indicate the critical role of IL-17R signaling during progression from an acute, macrophage-driven joint inflammation to a chronic, cartilage-destructive, T cell-mediated synovitis. There are four additional receptor-like molecules that share homology to IL-17R, i.e., IL-17Rh1 (also named IL-17RB or IL-17BR), IL-17RL (also named IL-17RC), IL-17RD, and IL-17RE. IL-17Rh1 was shown to bind to IL-17B, but with higher affinity to IL-17E (11, 12).

Although IL-17A transgenic mice have been reported to be embryonic lethal (39), we established BM-overexpressing mice that constitutively expressed IL-17A. The adequate control of the expression level was critically important. In our experiment, the serum concentration of IL-17A was elevated to ~600 pg/ml in IL-17A BM chimeric mice. This serum concentration of IL-17A was similar to those in patients with inflammatory diseases such as RA, inflammatory bowel diseases, familial Mediterranean fever, and the acute stage of Kawasaki disease (3, 44–46). Therefore, our BM chimeric mice approach may be useful to elucidate the physiological role of inflammatory cytokines that show lethal phenotypes in the conventional gene-transgenic technique.

In conclusion, we found that IL-17 family genes were up-regulated in association with their receptors in CIA. Each of the IL-17 family members clearly exacerbated the progression of CIA with the method of retrovirus-mediated BM chimeric mice. IL-17B and IL-17C have the capacity to exacerbate inflammatory arthritis in association with increased TNF-α and IL-6 productions from macrophages. Moreover, neutralization of IL-17B significantly suppressed the progression of arthritis and bone destruction in CIA mice. Therefore, our results suggest that not only IL-17A, but also the IL-17 family members IL-17B, IL-17C, and IL-17F play an important role in the pathogenesis of inflammatory arthritis and should be a new therapeutic target of arthritis.

Acknowledgments

Disclosures
The authors have no financial conflict of interest.

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関節リウマチに対する
インフリキシマブによる寛解導入療法

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Remission Therapy by Infliximab for Rheumatoid Arthritis

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Abstract

To analyze the course of remission in RA patients, after treatment with infliximab, we investigated 8 patients with previously established RA who had gone into remission and discontinued infliximab. Infliximab was discontinued when a negative CRP level, below 2.6 points of DAS 28, was continuously obtained for more than 6 months. Of the eight cases, one case (Stage Ⅲ) showed increased CRP levels 18 months after discontinuing infliximab. Another case (Stage Ⅱ) underwent complete remission without need of further infliximab or MTX 12 months after discontinuing infliximab. The other cases showed negative CRP, negative RF, and negative MMP-3 levels for 7.9 months on average after discontinuing infliximab. The MMP-3 levels significantly decreased to within normal range compared with those before treatment. Therefore, in remission after infliximab for early RA, not only prevention of further bone destruction but also increase in CRP levels were observed.

はじめに

関節リウマチ（以下RAと略す）の治療で関節変形をいかに抑制あるいは改善できるかは臨床上極めて重要である。関節変形は主に関節破壊から起こり，RAが発症してから約5年で75%が始まるとされている[1]。すなわち関節破壊を抑制するには早期治療が重要である。またRAには自然寛解があるとされているが実際のところ長期の詳細な報告はない。メトトレキサート（以下MTXと略す）を使用しても約3年で手指の変形は確実に進行することをしばしば経験する（図1）。このような内服薬のみの治療においてはRAの関節破壊は止められず，現在，

生物学的製剤をもってこの効果が発揮されることが明らかにされてきた[6]。RA発症早期に生物学的製剤を使用することにより関節破壊が防止可能である（Windows of Opportunity）[9]。2003年より日本に導入された生物学的製剤インフリキシマブによってその効果と副作用は明らかにされてきているが，日本人の寛解について

は不明である。インフリキシマブによって最も改善を示す関節は手指の関節であり，患者はインフリキシマブ投与後手指関節の疼痛，腫脹がとれるということが多い（図2）。寛解とはDisease Activity Score（以下DASと略す）28が2.6以下になるものを臨床的寛解とされているが[8]，RAの治療において薬剤中止可能すな
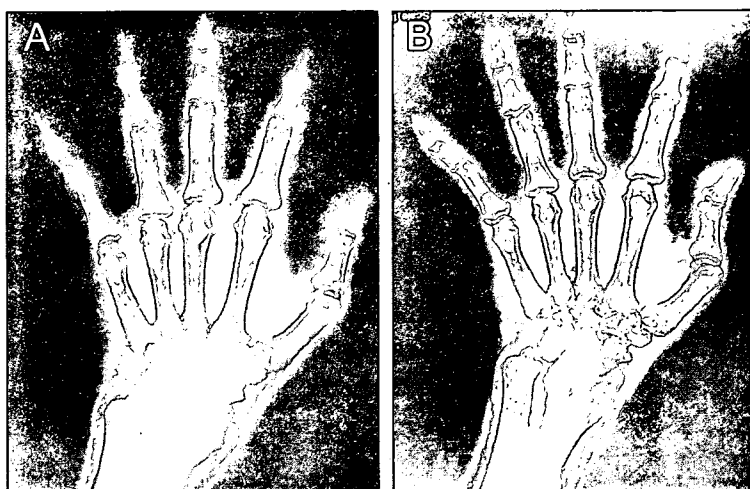

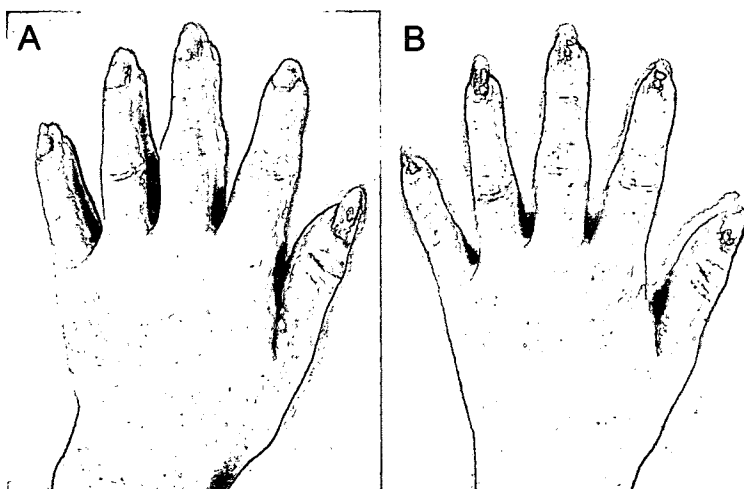図1　RAにおける関節破壊の進行，65歳　女性，
　　　MTX 6 mg にて治療した。A：治療前，B：治療後1年

図2　インフリキシマブによる手指関節腫脹の改善，43歳　女性，
　　　A：投与前，B：投与後1ヵ月

わちインフリキシマブ投与中止できた寛解は患者にとってより治癒に近い寛解とも言える。われわれはRAに対してインフリキシマブを使用した193例の患者背景とインフリキシマブを中止できた8例の臨床追跡評価とXP所見を検討したので報告する。

対象および方法

インフリキシマブ（レミケード，田辺三菱製薬）により当科で治療した関節リウマチは193例，男性31例，女性162例，Stage I 18例，Stage II 108例，Stage III 61例，Stage IV 6例で，Class I 8例，Class II 112例，Class III 69例，Class IV 4例であった。年齢は27歳から81歳，平均60.1歳であった。そのうち寛解症例は8例，男性3例，女性5例，Stage I 1例，Stage II 6例，Stage III 1例で，Class I 1例，Class II 8例であった。中止できた寛解症例の罹患期間は7ヵ月から372ヵ月，平均71.4ヵ月であった。MTXは4mgから6mg，平均5.25mg，ステロイド（predonine）は0mgから6mg，平均2.125mgであった。投与時のC-reactive protein（以下CRPと略す）は1.36mg／dlから4.5mg／dl，平均2.68mg／dlであった。リウマチ因子（以下RAPAと略す）は40から160で，平均100であった。MMP-3は51.3ng／mlから167ng／mlで，平均98.6ng／mlであった。インフリキシマブ継続期間は7ヵ月か

ら29ヵ月，平均13.1ヵ月で中止した。インフリキシマブ投与中止してから経過観察期間は症例1，6ヵ月，症例2，5ヵ月，症例3，7ヵ月，症例4，13ヵ月，症例5，18ヵ月，症例6，4ヵ月，症例7と8は5ヵ月であり，投与中止後平均経過観察期間は7.9ヵ月であった。インフリキシマブ投与中止の基準は投与開始してCRPが陰性化（当科では0.21mg／dl以下）してから最低6ヵ月以上経過し，DAS28が2.6以下の症例を中止した。中止後はMTX 4mg／週で治療し，症例4は中止後12ヵ月でMTXも中止している。インフリキシマブ投与全体の患者背景を調べるため，継続率および副作用や効果減弱にて脱落する因子をCox回帰分析を用いて解析した。投与後2週でのCRP改善を相関係数を用いて解析した。インフリキシマブ投与中止寛解症例の比率および早期RAにおける寛解比率を計算した。さらにインフリキシマブ投与中止寛解症例のMMP-3の改善をWilcoxonの符号付き順位検定を用いて比較した。インフリキシマブ投与中止寛解症例のCRPの投与前から中止後の推移とDAS28の変化および投与薬剤の変化を調べた。また寛解症例の手XP変化を比較検討した。

結果

インフリキシマブ投与して2週間後にCRPは0.55倍に減少した。すなわちCRP＝0.5469x

表1　インフリキシマブを中止できた寛解症例

症例数	症例	性別	年齢	Stage	MTX (mg)	PSL (mg)	体重 (kg)	身長 (cm)	BMI	CRP (mg/dl) 投与前	RAPA	MMP-3 ng/ml (投与前)	罹患期間 (月)	継続期間 (ヵ月)
1	K.S.	女性	57	II	6	0	55	152	23.81	1.36	40	64.60	47	10
2	T.A.	女性	54	I	6	0	50	156	20.55	1.63	160	53.70	24	7
3	S.M.	女性	81	III	4	1	46	148	21.00	1.88	160	N.A.	372	10
4	Y.H.	女性	52	II	6	0	46	157	18.66	4.01	40	86.4	22	18
5	M.M.	女性	72	III	4	0	53	153	22.64	1.54	160	51.3	7	29
6	M.N.	男性	69	II	6	5	71.9	170	24.88	4.50	40	167	21	11
7	D.M.	男性	27	II	4	6	90	160	35.16	2.64	40	157	14	9
8	S.T.	男性	56	II	6	5	75	167	26.89	3.87	160	110	40	11

（投与前CRP）であり投与後 2 週のCRPは投与前CRPに有意に相関していた（R2＝0.4731）（図 3）。インフリキシマブで寛解に入った症例は2004年 9 月15日から2006年 7 月22日までの間にインフリキシマブ投与した68例中 8 例11.8％寛解であり，そのうち早期RAでは28例中 7 例寛解であり寛解率25％であった。すなわち早期RAは全体と比較して約 2 倍の寛解率を示した。

76例のインフリキシマブ投与の投与継続率はKaplan-Meier法で 1 年，82％，2 年で71％，3 年で48％であった（図 4）。この継続に関する因子を，投与前CRP，投与後CRP，年齢，罹患期間，MTX量，ステロイド量についてCox回帰分析を行ったところ投与前CRP（p＝0.041）と年齢（p＝0.034）が有意に関係を示した（表 2）。このことは投与前CRPが高
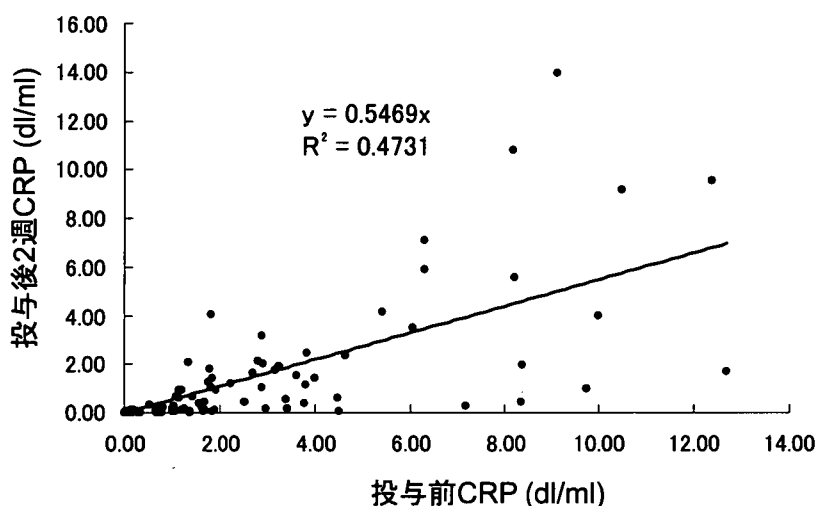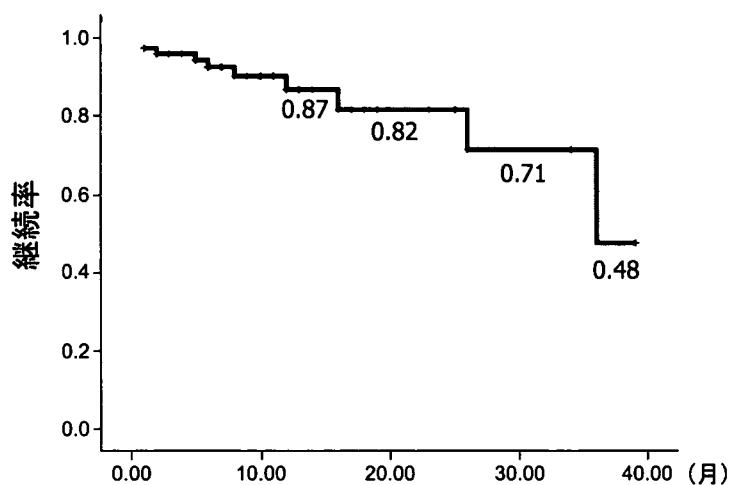

$$y = 0.5469x$$
$$R^2 = 0.4731$$

図3　投与後CRPの早期改善度

図4　インフリキシマブの継続率，Kaplan-Meier法（N＝76）