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T Cell Receptor Gene Therapy for Autoimmune Diseases

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ABSTRACT: **The current quality of autoimmune disease treatments is not satisfactory in regard to efficacy and safety. Antigen-specific immunotherapy is a future therapy that could achieve maximal efficacy with minimal adverse effects. T cells are essential components in antigen-specific immunity. However, we do not have a sufficient strategy for manipulating antigen-specific T cells. We propose that T cell receptor (TCR) gene transfer is a hopeful approach for antigen-specific immunotherapy. We confirmed the efficacy of TCR gene therapy in animal models of systemic autoimmune disease and arthritis. In lupus-prone NZB/W F1 mice, nucleosome-specific TCR and CTLA4Ig transduced cells suppressed autoantibody production and nephritis development. In the therapeutic experiment of collagen-induced arthritis (CIA), arthritis-related TCRs were isolated from single T cells accumulating in the arthritis site. Arthritis-related TCR and TNFRIg transduced cells or TCR and Foxp3 transduced cells suppressed arthritis progression and bone destruction. Therefore, engineered antigen-specific cells manipulated to express appropriate functional genes could be applied to specific immunotherapy.**

KEYWORDS: **autoimmune diseases; antigen-specific T cells; gene transfer; T cell receptor**

INTRODUCTION

Rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes are regarded as diseases associated with autoimmunity. These autoimmune diseases are relatively common disorders affecting about 5% of the population, predominantly women.[1] Current treatment of the autoimmune diseases is composed of nonspecific immunosuppressive drugs, such as corticosteroids and cytotoxic reagents. Though nonspecific immunosuppressive therapy has improved clinical outcome of patients in autoimmune diseases, it is

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accompanied by several serious adverse effects. Since cytokines play a pivotal role in immune reactions, application of cytokines has been extensively studied to control autoimmune diseases. Systemic administration of suppressive cytokines, such as transforming growth factor (TGF)-beta, interleukin (IL)-4, and IL-10 showed a significant efficacy in models of autoimmune diseases. Some of these agents seem to work by shifting the balance of immune deviation. However, systemic cytokine therapy potentially leads to deleterious side effects, as in the case of recombinant human IL-4.[2] On the other hand, cytokine-blocking therapies have been successful and will continue to serve as important strategies in many autoimmune diseases. However, because such strategies still have important drawbacks, including severe infections,[3] it will also be necessary to explore other specific immunotherapies.

Autoantigens for autoreactive T cells and autoantibodies have been extensively explored in various autoimmune diseases. These explorations are important not only for understanding the pathogeneses of autoimmune diseases, but also for establishing antigen-specific immunotherapies. If possible, suppression of the initial activation of antigen-specific T cells is feasible because intervention appears to be less effective on established pathogenic T cells. However, the majority of patients who require clinical treatment have full-blown autoimmune disease, and this approach would not be adequate. In advanced autoimmune diseases, an immune response to a single epitope on a self-antigen at the start of the disorder can trigger immune responses to adjacent epitopes on the same molecule or to other epitopes on related molecules. This phenomenon is called "epitope spreading." Although the precise impact of the epitope spreading is not evident in the entire autoimmune process, some researchers argue against antigen-specific immunotherapy because of the difficulties of predicting such expanding autoimmune reactions. However, we propose that epitope spreading is not the sole mechanism of the T cell–related pathogenesis of autoimmune diseases and that clonal restriction of T cells occurs in the late phase of autoimmunity. In this context, antigen-specific immunotherapy would be feasible, even for established autoimmune diseases. T cell receptor (TCR) gene transfer could be one of the possible strategies.

EVALUATION OF ANTIGEN-SPECIFIC T CELLS IN AUTOIMMUNE DISEASES

The model of epitope spreading or determinant spreading has been generally accepted in autoimmunity.[4,5] With respect to T cells, this is a diversification of specificity from the initial limited epitope-specific immune response to a hierarchical cascade of autoreactive T cell specificities. This model could explain the pathway of infection-induced autoimmunity. On the basis of this idea, the initial phase of the autoimmune reaction might be invoked by a few activated T cells against limited numbers of epitopes. These T cells may

be cross-reactive to both microbial epitopes and self-epitopes. On the other hand, in the late phase of the disorders, the reactive epitopes might spread and T cells recognizing a variety of different epitopes on the several different self-molecules would be activated. However, if epitope spreading is the only mechanism involved in the T cell immune responses in autoimmune disorders, development of effective antigen-specific immunotherapies will be difficult, because target epitopes and molecules will always have the potential to spread, and it would be difficult to define the pattern of spreading in a chronic human autoimmune disorder.

To verify the presence of epitope spreading throughout the autoimmune process, it is important to detect how specific T cells behave within the lymphocyte population in the pathological lesions. Previously, our group established a method to analyze accumulated T cell clones using RT-PCR and single-strand conformation polymorphism (SSCP) on TCR messages.[5] With this method, the same clones were found to exist in different joints, of an RA patient.[5,6] These results clearly suggested the uniformity of immune responses in RA throughout the arthritic lesions. In the case of HTLV-1 env-pX transgenic mice, which exhibit spontaneous symmetrical arthritis similar to human RA,[7] there were vigorous accumulations of T cells in the joints, but they were different among the different lesions in the early stage.[8] In the middle stage, several identical clones were accumulated in the different lesions. In the late stage, the majority of the accumulated clones exist uniformly in several arthritic lesions. The number of the dominant clones did not necessarily increase. These results in mice also suggested the relative uniformity of autoimmune responses in the pathological lesions.

We have observed similar clonal restriction in several spontaneous autoimmune animal models.[9,10] Moreover, oligoclonally expanded insulin-reactive T cells were identified in the pancreatic draining lymph nodes from type 1 diabetes patients with prolonged disease durations.[11] A limited T cell oligoclonality as a "driver clone" in autoimmunity was described in experimental autoimmune encephalomyelitis (EAE).[12,13] In polymyositis patients, several T cell clones persisted for several years in blood T lymphocytes and consecutive muscle biopsy specimens.[14] On the basis of these observations, we now speculate that epitope spreading does not necessarily work in the late phase of the disease progression, and it is possible that some form of clonal restriction of T cells occurs in autoimmune disorders. Some restricted T cell clones directed toward certain target self-antigens might be sustained. Avidity maturation of a pathogenic T cell population may be the decisive event in the progression of benign inflammation to full-blown autoimmune disease.[15] Therefore, it is feasible to suppress sustained pathologic responses without global immuosuppression.

In immune responses to foreign antigens, T cell responses are dominated by few clonotypes.[16] This clonal selection and dominance may be due to the competitive advantages of higher-affinity receptor, duration of TCR–pMHC

interaction, or affinity threshold.[17] In viral infections, clonal T cell "immunodomination" occurs in CD8+ T cells, probably because of proliferation advantages, differences of TCR affinity, or co-signal requirements.[18] Hence, the clonal restriction of T cells is not a behavior specific to these diseases, but can be considered as an universal phenomenon.

TCR GENE TRANSFER FOR CONTROLLING AUTOIMMUNE DISEASES

In the application of T cell targeted antigen-specific immunotherapy, there are several technical difficulties in the establishment of autoantigen-specific T cells. Usually the culture should be performed without the information of appropriate autoantigens. We have to select a candidate autoantigen in cloning culture of autoantigen-specific T cells based on the limited information. Moreover, there is no guarantee that *in vitro* established T cell clones represent real disease-associated T cells, mainly because *in vivo* activated T cells are more easily rendered in activation-induced cell death. Therefore, we attempted to produce inflammation-associated T cells by gene transfer of TCRs obtained *in vivo*.

Reconstitution of Antigen-Specific T Cells By TCR Gene Transfer

TCRs of the accumulated T cell clones in the inflammatory lesions can be visualized by RT-PCR/SSCP analysis. We attempted to obtain a pair of full-length cDNAs encoding alpha and beta chains of TCR expressed in a single cell in the lesion. We can reconstitute TCR function by expressing them with gene transfer to self T cells. For the gene transfer to lymphocytes, we have established a highly efficient retrovirus vector system with PLAT-E and pMX. PLAT-E is a packaging cell transfected gag–pol and env segment separately. Two independent monocistronic retrovirus vectors harboring alpha and beta TCR cDNAs were generated. For the first study, the class II MHC-restricted alpha and beta TCR genes specific for chicken OVA were used. These TCR genes were cloned from TCR transgenic mice designated DO11.10. These TCRs were transduced to splenocytes from BALB/c mice. The results indicated that alpha and beta TCR gene transfer into peripheral T cells reconstituted the antigen-specific immunity.[19] The amount of TCR expression and both the *in vitro* and *in vivo* antigen-specific functions were comparable to those obtained with splenocytes from DO11.10 transgenic mice. Moreover, DO11.10 TCR and IL-10-co-transduced CD4-positive T cells suppressed delayed type hypersensitivity to OVA, strongly compared to IL-10-transduced polyclonal CD4-positive T cells (Okamura *et al.*, unpublished observation).

Gene Therapy of a Model of Systemic Autoimmune Disease

We next attempted to use this TCR gene transfer to control autoimmune disorders. The target was lupus-prone NZB/W F1 mice, which spontaneously develop a lupus-like syndrome and nephritis. Anti-DNA antibodies are believed to be one of the major pathogenic autoantibodies for the nephritis. Datta and others have pointed out that nucleosome is a major immunogen in SLE.[20,21] Since DNA and nucleosome are physically associated, it is speculated that nucleosome-reactive T cells help the activation of anti-DNA-specific B cells as the hapten-carrier model. Therefore, we tried to generate nucleosome-specific T cells with an immunosuppressive function.[22] We selected CTLA4Ig as a suppressive molecule. TCR cDNAs were engineered on the basis of the published sequence of nucleosome-specific TCRs by fusing TCR V region sequence, synthesized CDR3 sequence, and TCR J-C region sequence. They were V alpha 13 and V beta 4. This TCR recognizes the immunodominant I-A^d-restricted nucleosomal epitope.

In our usual experimental protocol, the proportion of clonotypic TCR expression cells with two transferred TCR genes was estimated to be about 25% in CD4 + T cells. The introduction of TCR was found to reconstitute the specificity for the nucleosome. We then performed triple gene transfer together with CTLA4Ig to generate regulatory T cells (FIG. 1). Our calculations showed that approximately 10% of the total CD4-positive cells expressed all three genes. The CTLA4Ig secreted from transduced T cells blocked the proliferation of the polyclonal T cell population. The TCR and CTLA4Ig transduced cells showed the increase of CTLA4Ig secretion on T cell activation in the presence of DCs. A million of the nucleosome-specific regulatory T cells engineered by the triple genes were then transferred into 10-week-old NZB/W F1 mice. The mice were monitored for proteinuria. By week 22, all of the control mice that had received PBS, cells transferred with mock vectors, TCR alone, and CTLA4Ig started to develop severe nephritis diagnosed by the presence of persistent proteinuria of more than 300 mg/dL. By 30 weeks of age, the majority of these control mice showed severe proteinuria. However, none of the mice treated with cells transferred by the TCRs and CTLA4Ig showed excess proteinuria. The kidneys of the control mice showed severe glomerulonephritis with membranoproliferation, glomerular sclerosis, and tubular casts. The treated mice had mild glomerular disease with less deposition of IgG and complement, especially in the capillary loop. The autoantibodies usually found in NZB/W F1 mice were measured in the sera from different groups. The elevations of anti-dsDNA and anti-histone antibodies were suppressed at 22 weeks of age in the TCR and CTLA4Ig-treated mice. The T cell–dependent humoral response to active immunization of OVA was also analyzed. The level of anti-OVA IgG antibody titer was not significantly different from those of the control mice, indicating there was not an overt systemic immunosuppression of the triple gene-treated mice.

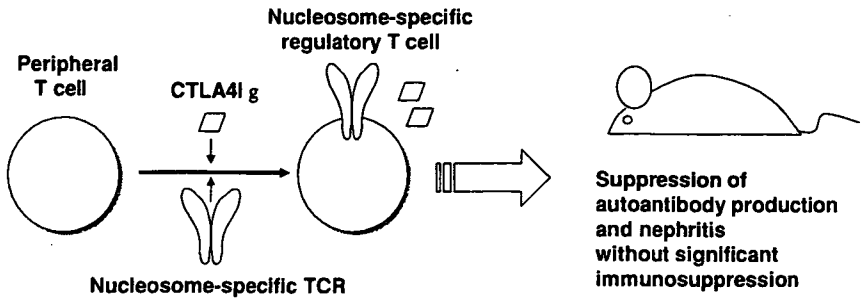

FIGURE 1. Experimental outlines of TCR gene transfer for systemic autoimmune diseases. Therapeutic effect of nucleosome-specific CTLA4Ig-producing T cells in lupus-prone NZB/W F1 mice is shown.

Gene Therapy of Arthritis with Inflamed Paw Homing TNFRIg-Producing T Cells

In order to obtain the whole TCR information from pathological lesions, we developed a method to clone a pair of full-length TCR cDNAs from a single cell accumulated in the inflamed joints of DBA/1 mice with collagen-induced arthritis.[23] Cloning of full-length cDNA encoding TCR was already established.[24] Single cell sorting with CD4+ and V beta 8.1/8.2-positive cells was performed and TCR messages were amplified with 3-step nested PCR using a fixed V beta primer and multiple V alpha primers. We then compared the clones obtained from the single cells with accumulated clones observed in the arthritic joints using the RT-PCR/SSCP method. Some TCRs from sorted single cells were actually identical to major accumulated clones in the joints. The full-length TCR cDNAs were subcloned into retrovirus vectors and transferred to DBA/1 splenocytes. Interestingly, some of the pairs of TCR were found to be not specific to immunized type II collagen, but reactive to self-antigen, because TCR-transferred cells proliferated in the culture with DCs from normal and arthritic mice. The carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling experiments showed that such TCR-transduced cells accumulated and proliferated in the arthritic joints. We next performed a therapeutic experiment using the triple gene-engineered T cells. In this experiment, soluble fusion protein of TNF receptor p75 and Fc domain of IgG2a (TNFRIg) was used as a regulatory molecule. We selected one of the TCR pairs, B47, which expanded in the arthritic paws. Control cells were transduced with either B47 alone or TNFRIg alone. In terms of the arthritis score as well as the incidence of severe arthritis, only B47 plus TNFRIg-transduced cells significantly suppressed the arthritis (FIG. 2A). Interestingly, the serum concentration of TNFRIg was not the main determinant of arthritis suppression in the B47+TNFRIg group, because the serum concentrations of TNFRIg protein in the B47+TNFRIg group were equivalent to those in the TNFRIg group. In contrast, the amount

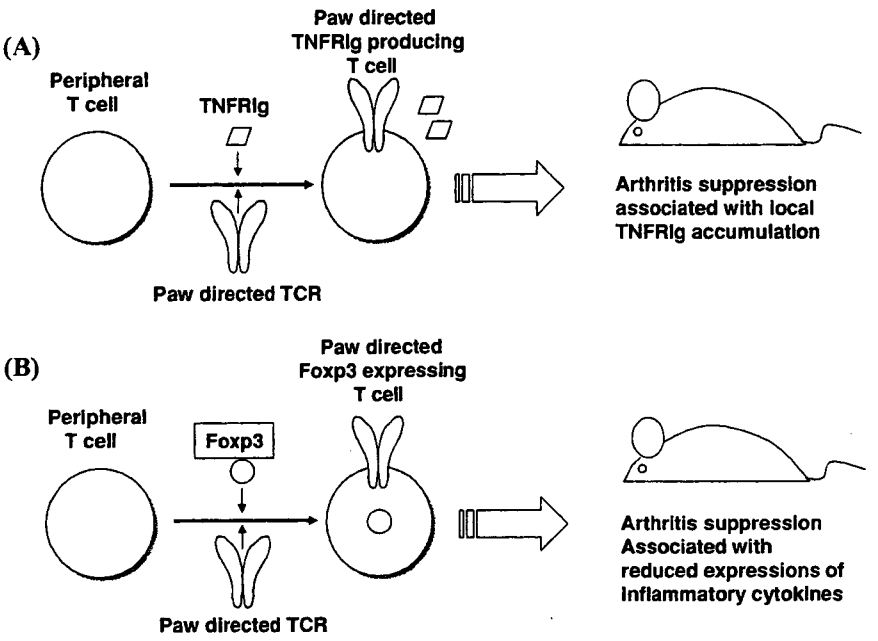

FIGURE 2. Experimental outlines of TCR gene transfer for collagen-induced arthritis. (**A**) and (**B**) illustrate therapeutic effect of paw-directed TNFRIg-producing T cells and paw-directed Foxp3-expressing T cells.

of TNFRIg in the paws of the B47+TNFRIg group was significantly higher than that in the paws of the TNFRIg group. Therefore, local accumulation of the TNFRIg transcript suppressed arthritis in the B47+TNFRIg group, and so biological agents producing T cells may have the advantage over the conventional biological agents that depend on serum concentration. A reduced serum concentration may be associated with less systemic immunosuppression.

Gene Therapy of Arthritis with Inflamed Paw Homing Foxp3-Expressing T Cells

Fopx3 is reported to be the key regulator for regulatory T cells. Several groups have reported that regulatory T cells are accumulated in the joints of arthritis patients.[25,26] Joint accumulating $CD4^+CD25^+$ T cells displayed an increased suppressive capacity compared with blood $CD4^+CD25^+$ T cells. However, the precise role of these accumulating regulatory T cells was not clarified in arthritis pathology. We generated B47+Foxp3 transduced cells and three groups of controlled gene transfer, Mcok, B47 alone and Foxp3 alone. The reconstituted regulatory T cell group, B47+Foxp3, significantly

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.

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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

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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.

Dr. Yamamoto reports having equity interest in ImmunoFuture. No other potential conflict of interest relevant to this article was reported.

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

Yumi Yamaguchi,* Keishi Fujio,[2]* Hirofumi Shoda,* Akiko Okamoto,* Nelson H. Tsuno,† Koki Takahashi,† and Kazuhiko Yamamoto*

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

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[3] Abbreviations used in this paper: RA, rheumatoid arthritis; BCII, bovine type II collagen; BM, bone marrow; CIA, collagen-induced arthritis; mIL, murine IL; MMP, matrix metalloproteinase; PEC, peritoneal exudate cell; pMIG, murine stem cell virus/internal ribosome entry site/GFP.

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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. 1*A*). 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. 1*B*).

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. 1*C*). 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. 2*A*).

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. 2*B*). 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. 2*C*). These results suggested that IL-17A, IL-17B, IL-17C, and IL-17F stimulate fibroblasts and macrophages to produce inflammatory cytokines.

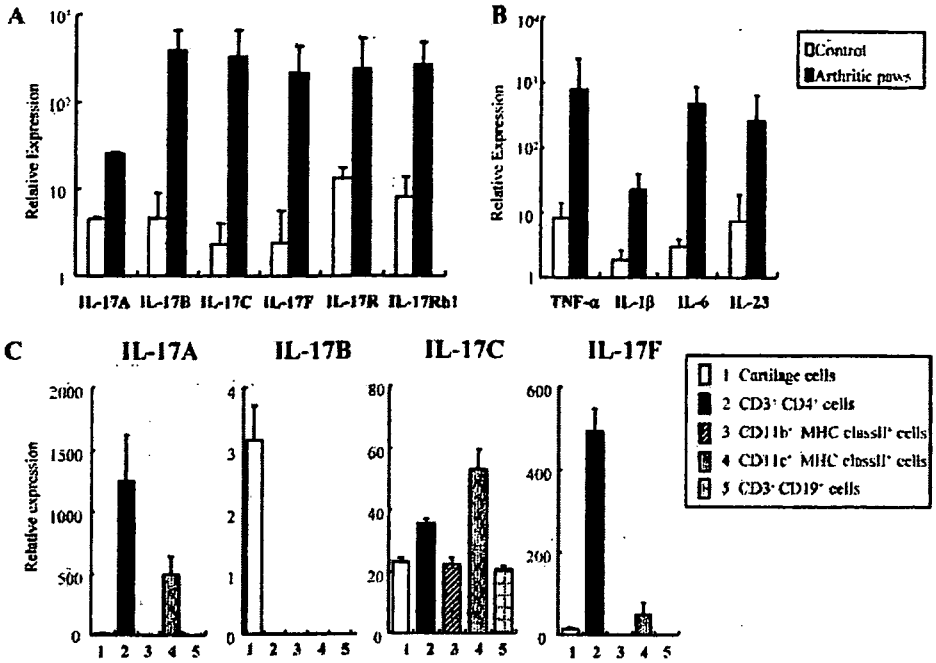

FIGURE 1. The expression of IL-17 family members and IL-17R genes in the arthritic paws of CIA mice. *A*, The expressions of IL-17 family genes and IL-17R genes were examined in the arthritic paws of CIA mice (■; $n = 3$) and in control mice (□; $n = 3$) by quantitative PCR. *B*, The expressions of inflammatory 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

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. 3*A*).

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. 3*B*). 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

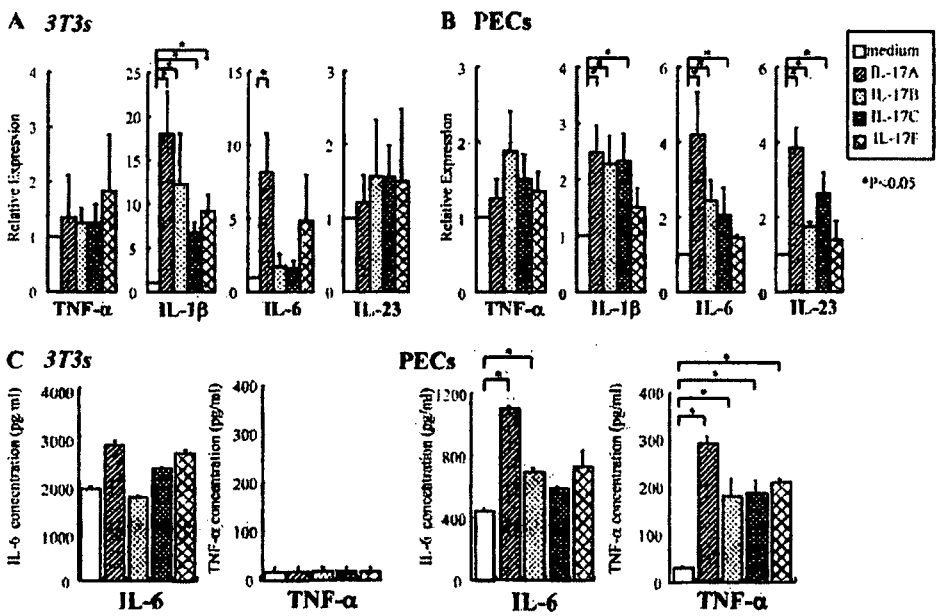

FIGURE 2. The proinflammatory effects of IL-17 family members on mouse fibroblasts and macrophages. *A*, Relative expression of the cytokine genes in *3T3* cell. The mouse fibroblast cell line *3T3* was cultured with each of mIL-17A, mIL-17B, mIL-17C, or mIL-17F for 24 h, and the expressions of inflammatory 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: *, $p < 0.05$.

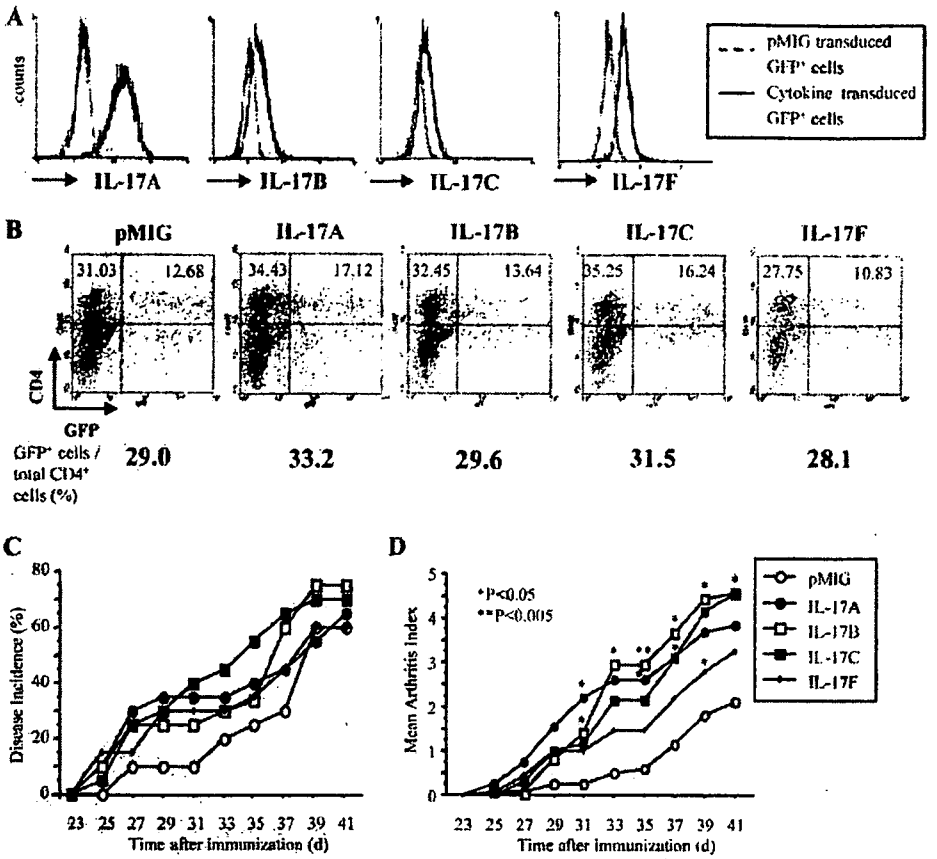

FIGURE 3. The effects of transfer of IL-17 family-transduced CD4$^+$ T cells on CIA. *A*, Intracellular IL-17 family expressions in Ba/F3 cells retrovirally transduced with each IL-17 family member. GFP-gated IL-17 family-transduced (mIL-17A, mIL-17B, mIL-17C, or mIL-17F) Ba/F3 cells were analyzed for 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 expression 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.

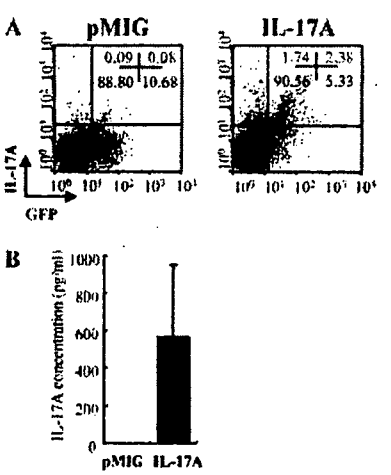

FIGURE 4. Generation of IL-17 family chimeric mice by BM transplantation of gene-transduced BM cells. Each of IL-17 family genes was transduced to BM cells with retrovirus vector and transferred to lethally irradiated mice. *A*, The intracellular expression of IL-17A protein in the spleen of IL-17A BM 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.