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Suppression of Immune Induction of Collagen-Induced Arthritis in IL-17-Deficient Mice

Susumu Nakae, Aya Nambu, Katsuko Sudo, and Yoichiro Iwakura

Interleukin-17 is a T cell-derived proinflammatory cytokine. This cytokine is suspected to be involved in the development of rheumatoid arthritis (RA) because this cytokine expression is augmented in synovial tissues of RA patients. The pathogenic roles of IL-17 in the development of RA, however, still remain to be elucidated. In this study, effects of IL-17 deficiency on collagen-induced arthritis (CIA) model were examined using IL-17-deficient mice (IL-17 knockout mice). We found that CIA was markedly suppressed in IL-17 knockout mice. IL-17 was responsible for the priming of collagen-specific T cells and collagen-specific IgG2a production. Thus, these observations suggest that IL-17 plays a crucial role in the development of CIA by activating autoantigen-specific cellular and humoral immune responses. The Journal of Immunology, 2003, 171: 6173-6177.

Rheumatoid arthritis (RA) is one of the most serious medical problems, affecting ~1% of all people worldwide, irrespective of race. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissues in multiple joints that leads to joint destruction, but the etiopathogenesis has not been elucidated completely.

Various disease models for RA have been developed, and collagen-induced arthritis (CIA) is one of the well-established models. CIA can be induced in susceptible rodents by intradermal injections of homologous or heterologous native type II collagen (IIC), a major component of cartilage Ags. Susceptibility for the disease is dependent on MHC class II haplotypes, and only mice with H-2b and H-2r haplotypes respond to immunization with IIC and develop arthritis. Recently, however, it is reported that CIA can also be induced in C57BL/6 (H-2b) mice by repeated administration with IIC intradermally (4-6). The development of CIA is dependent on both cellular and humoral immune responses to IIC (6), and various cytokines are thought to play crucial roles in the pathogenesis through the activation of immune system (6, 7).

A T cell-derived proinflammatory cytokine, IL-17 is produced by TCRαβ CD4 CD8 thymocytes, as well as activated CD4+ and CD4+CD45RO+ memory T cells (8). The producer cells of IL-17 also express TNF-α, but not Th1 or Th2 cytokines, in mice (9). IL-17 has pleiotropic activities, including induction of TNF-α, IL-1β, IL-6, IL-8, G-CSF, and monocyte chemoattractant protein-1 on various types of cells (8).

Involvement of IL-17 in the development of RA is suggested, because IL-17 is found in the synovial fluid of RA patients (10) and is produced by T cell clones established from RA patients (11). Actually, the incidence of arthritis reduced partially by the administration of an extracellular domain of IL-17R-Fc fusion protein (IL-17R-Fc), which inhibits IL-17-IL-17R binding, in the elicitation phase during CIA (12). The precise role for IL-17 in the pathogenesis of RA, however, still remains to be elucidated.

In this study, using IL-17 knockout mice, we assessed the role of IL-17 in the development of CIA. The incidence of CIA was markedly suppressed in IL-17 knockout mice, and a crucial role for IL-17 was suggested in activating collagen-specific T and B cells during CIA.

Materials and Methods

Mice

IL-17 knockout mice were generated, as described previously, using E14.1 ES cells (13). For CIA induction, IL-17 knockout mice on (129/Sv × C57BL/6)F1 hybrid background were used, and the IL-17−/− littermates were used as the controls. These mice were kept under specific pathogen-free conditions in an environmentally controlled clean room in the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo. The experiments were conducted according to the institutional ethical guidelines for animal experiments and the safety guideline for gene manipulation experiments.

Collagen-induced arthritis

CIA in mice on 129 × B6 F1 hybrid background was performed, as described elsewhere (5). Briefly, mice were immunized with 100 μl of 1 mg/ml chicken IIC (Sigma-Aldrich, St. Louis, MO) emulsified with CFA intradermally at several sites into the base of the tail. CFA was prepared by the mixture of 100 mg heat-killed Mycobacterium tuberculosis (H37Ra; Difco Laboratories, Detroit, MI) and 20 ml IFA (Difco). At 21 days after the first immunization, mice were again challenged with collagen/CFA nearby the primary injection site intradermally.

Clinical and histological assessment of arthritis

Development of arthritis by macroscopic evaluation was determined, as described elsewhere (14). At 60 days after the first immunization during CIA, limbs were fixed with 10% neutral Formalin and were decalcified with 5% formic acid. They were embedded in paraffin, and 5-μm slices were prepared. Sections were stained with H&E.

T cell culture

For IIC-specific T cell proliferative response, inguinal lymph nodes (LN) were harvested from mice at 1 wk after the primary immunization with IIC/CFA. Single cell suspension was prepared, and LN cells (3 × 10⁶ cells/well) were cultured in the absence of presence of 50 μg/ml denatured chicken IIC for 72 h, followed by incorporation of [3H]thymidine (0.25 μCi/ml) (Amersham, Chalfont St. Giles,

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2 Abbreviations used in this paper: RA, rheumatoid arthritis; CHS, contact hypersensitivity response; CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalomyelitis; IIC, type II collagen; LN, lymph node.
Buckinghamshire, U.K.) for 6 h. Then cells were harvested with a Micro 96

cell harvester (Skatron, Lier, Norway), and radioactivity was measured with

Micro Beta (Pharmacia Biotech, Piscataway, NJ).

Detection of cytokine by ELISA
IL-17 levels were measured by ELISA, as described previously (13). Monoclonal rat anti-mouse IL-17 and polyclonal biotinylated goat anti-
mouse IL-17 Abs (DAKO, Carpentry, CA) were used as a capture and
detection Ab, respectively. HRP-avidin was obtained from BD PharMin-
gen (San Diego, CA), and a tetramethylbenzidine One-Step Substrate Sys-
tem was obtained from DAKO. rIL-17 as a standard reagent was obtained
from Sigma-Aldrich. To measure IL-4 and IFN-γ levels, BD OptiELA
ELISA Sets were purchased from BD PharMingen.

Measurement of collagen-specific Ig titers
Sixty days after the first and second immunizations with IIC/CFA, serum
was collected. A total of 10 µg/ml IIC in PBS was coated on Falcon 3912
Micro Test III Flexible Assay Plates (BD Biosciences, Oxnard, CA) at 4°C
overnight. After washing with PBS, serially diluted serum samples were
applied and incubated at room temperature for 1 h. Then the wells were
washed with PBS + 0.05% Tween 20, followed by the addition of alkaline
phosphatase-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, and IgG3
(Zymed, San Francisco, CA). Alkaline phosphatase activity was measured
using Substrate Phosphatase SIGMA104 (Sigma-Aldrich) as the substrate.

Results

Suppression of development of CIA in IL-17−/− mice
To assess the role of IL-17 in the development of arthritis, the effect of IL-17 deficiency on the development of CIA was exam-
ined. As shown in Fig. 1A, the incidence of the disease in IL-17−/−
mice was markedly suppressed compared with that in IL-17+/+ mice.
Likewise, the severity score in IL-17−/− mice was also milder than that in IL-17+/+ mice (Fig. 1B). A histological anal-
ysis of the joints of IL-17+/+ mice immunized with IIC showed

typical features of arthritis, which was characterized by marked
synovial and periarticular inflammation with extensive polymor-
phonuclear cell infiltration, synovial hyperplasia, and bone erosion
(Fig. 2, C and E). The articular bone and cartilage were invaded
with granulation tissues forming a pannus, and degenerative prod-
ucts of the bone structure were found in the articular cavity. In
contrast, the joint pathology of IL-17−/− mice revealed much
milder inflammation with profoundly reduced cell infiltration, and
synovial hyperplasia and bone erosion were also suppressed,
indicating both inflammatory and destructive features of the joints
were suppressed in IL-17−/− mice (Fig. 2, D and F). These results
indicate that IL-17 is involved in the development of CIA.

Reduced Ab production against IIC in IL-17−/− mice
Because Ab levels against IIC correlate well with the development
of arthritis (15), we examined the development of IIC-specific Abs in
IL-17−/− mice. Sera were collected 60 days after the first
immunization with chicken IIC/CFA, and IIC-specific Ab levels were
measured by ELISA. Collagen-specific IgG2a levels in sera from
IL-17−/− mice were significantly lower than those from IL-17+/+ mice,
whereas the levels of other IgG subclass Abs were not different
between IL-17+/+ and IL-17−/− mice (Fig. 3). These results indicate
that IL-17 is involved in collagen-specific Ab production during CIA.

Reduced IIC-specific T cell responses in IL-17−/− mice
To elucidate the role of IL-17 in T cell function, we examined T
cell response in IL-17−/− mice. A T cell Ag-recalling assay
against IIC was conducted 1 wk after the first immunization with
chicken IIC/CFA. Proliferative response against IIC of LN cells
from IL-17−/− mice was significantly reduced compared with that
from IL-17+/+ mice (Fig. 4A), showing that IL-17-deficient T cells
are sensitized incompletely. During the incubation, a significant
amount of IL-17 was secreted in the supernatants of IL-17+/+ LN
cell cultures, but no production was detected in IL-17−/−
cell cultures (Fig. 4B). IFN-γ production after stimulation with IIC was
markedly reduced in IL-17−/− LN cell culture, while IL-4 produ-
c tion was almost undetectable both in the IL-17+/+ LN cell

culture and IL-17−/− T cell culture (Fig. 4B). These results
indicate that IL-17 plays an important role in the Ag-specific T cell
activation during priming phase of CIA.

Discussion
In this study, we showed that, by using IL-17−/− mice, IL-17 is

crucial for the activation of autoantigen-specific T and B cells in
the sensitization phase of CIA. Various inflammatory cells includ-
ing T cells are found to infiltrate the local inflammatory site of RA
patients, and it has been believed that the T cell-derived Th1 cyto-
kine is involved in the pathogenesis of RA. However, IFN-γ, a
major Th1 cytokine, has been considered to play rather bene-
cial roles (16–18). In contrast, inflammatory cytokines, TNF-α and
GM-CSF, which are also produced by CD4+ T cells (9), are sug-

gested to have an important role in the RA pathogenesis. Indeed,
the development of CIA in TNF-α−/− mice (C57BL/6 background)
was milder than that of wild-type mice, and markedly
suppressed in GM-CSF−/− mice (backcrossed to C57BL/6 for 11

FIGURE 1. The reduced development of CIA in IL-17−/− mice. Mice were

immunized with chicken IIC emulsified with CFA intradermally at several

sites into the base of the tail on days 0 and 21 (arrow). Incidence (A) and severity (B) of CIA are shown. IL-17−/− mice, ■ (n = 13) and
IL-17+/− mice, ● (n = 12). Data obtained from two independent experi-
ments were combined and shown. Average and SD are indicated. *, p <
0.005 vs IL-17+/+ mice by χ2 test. †, p < 0.01, and ‡, p < 0.05 vs
IL-17+/+ mice by Mann-Whitney U test.

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Because both TNF-α and GM-CSF are produced by various cell types including fibroblasts, the suppressive effect of CIA may not solely be a result of their production by CD4⁺ T cells. However, in a recent study, it was reported that IL-17 is produced by CD4⁺ T cells that are coexpressing TNF-α and/or GM-CSF, but not by Th1 and Th2 cells (9). Thus, a certain T cell subset, coexpressing IL-17, TNF-α, and/or GM-CSF rather than Th1-producing IFN-γ, may have an important role in the pathogenesis of RA. Although it was believed that contact hypersensitivity response (CHS) is mediated by IFN-γ-producing Tc1 and Th1 cells (19), we showed that CHS was normal in IFN-γ⁻/⁻ mice (20), while it was markedly suppressed in IL-17⁻/⁻ mice (13), indicating that CHS as well as CIA are mediated by CD4⁺ T cells producing IL-17, but not IFN-γ. Moreover, experimental autoimmune encephalomyelitis (EAE) is also considered to be an IFN-γ-producing Th1 cell-mediated inflammatory autoimmune disease. However, many investigators have shown that IFN-γ⁻/⁻ and IFN-γR⁻/⁻ mice are highly susceptible to EAE compared with control mice, indicating that IFN-γ-producing Th1 cells are required for the protection of this disease (21–24). In contrast, IL-17 mRNA was increased in multiple sclerosis, suggesting that IL-17 is involved in the pathogenesis of multiple sclerosis (25, 26). In fact, in our unpublished observation, EAE in IL-17⁻/⁻ mice was significantly suppressed (Y. Komiyama, S. Nakae, and Y. Iwakura, in preparation). Thus, IL-17-producing T cells rather than IFN-γ-producing Th1 cells play critical roles in the pathogenesis of T cell-dependent autoimmune disease.

Recently, it was reported that the administration of IL-17R:Fc fusion protein to DBA/1 mice after secondary immunization with IIC/CFA suppresses the disease by blocking the IL-17/IL-17R binding, suggesting that IL-17 plays an important role in the inflammatory phase (12). In the present study, we have shown that IL-17 is involved in autoantigen-specific T cell priming and expansion (Fig. 4), although the roles of IL-17 in the inflammatory phase are not known in this experiment. Taken together, these observations indicate that IL-17 plays important roles not only in the induction of local inflammation of joints, but also in autoantigen-specific T cell activation in the priming phase.

**FIGURE 2.** Histological analysis in the joint of hind limbs during CIA. Sections of the joint of hind at 60 days after the first immunization during CIA were stained with H&E. The joint of nonimmunized IL-17⁺⁺ (A) and IL-17⁻⁻ (B) mice and that of IIC-immunized IL-17⁺⁺ (C and E) and IL-17⁻⁻ (D and F) mice. A–D, ×40; E and F, ×100.
As mentioned above, IL-17 is required for autoantigen- and collagen-specific T cell priming and Ab production in IL-17−/− mice (13). Yao et al. (27) also reported that T cell proliferation and IL-2 production induced by soluble IL-17R, indicating that IL-17 is involved in T cell priming and Ab production (Figs. 3 and 4).

**References**


cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. J. Immunol. 165:6783.


