Collagen-Induced Arthritis

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Mark Boothby and Jin Chen

\( J \text{Immunol}\) 1999; 163:1577-1583;

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The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Essential Role of T Cell NF-κB Activation in Collagen-Induced Arthritis

Rajalakshmi Seetharaman,* Ana L. Mora, † Gerald Nabozny, ‡ Mark Boothby, † and Jin Chen2*

NF-κB/Rel proteins are ubiquitous transcription factors that are activated by proinflammatory signals or engagement of Ag receptors. To study the role of NF-κB/Rel signaling in T lymphocytes during autoimmune disease, we investigated type II collagen-induced arthritis (CIA) in transgenic mice expressing a constitutive inhibitor of NF-κB/Rel (IxBα(ΔN)) in the T lineage. Expression of the IxBα(ΔN) transgene was persistently high in adult peripheral lymphoid organs and undetectable in T cell-depleted splenocytes, suggesting the expression of the transgene is restricted to the T lineage. The incidence and severity of CIA were decreased significantly in these IxBα(ΔN) transgenic mice compared with nontransgenic littermates. Inhibition of CIA was not due solely to a decrease in their CD8+ population because transfer of wild-type CD8+ cells into transgenic mice failed to restore disease susceptibility. Protection against disease was associated with a moderate decrease in clonal expansion and a profound and persistent decrease in Ag-induced IFN-γ production in vivo. Consistent with decreased level of anti-type II collagen-specific Abs and IFN-γ, serum levels of IgG2a anti-CII Abs were significantly reduced. However, anti-CII-specific IgG1 levels were normal, indicating that some aspects of T cell help were unaffected. Taken together, these results suggest that inhibition of NF-κB in T cells impairs CIA development in vivo through decreases in type 1 T cell-dependent responses. The Journal of Immunology, 1999, 163: 1577–1583.

Collagen-induced arthritis (CIA)3 is an animal model of autoimmune disease that has been extensively used to elucidate the pathogenic mechanisms relevant to human rheumatoid arthritis (reviewed in Refs. 1–3). The development of CIA is known to depend on T cell activation. Like human rheumatoid arthritis, the disease susceptibility of CIA is controlled by the MHC class II locus, being restricted to H-2q or H-2r haplotypes. Treatment with Abs to CD4, TCR, MHC class II, IL-2R, or CD28 at the time of immunization blocks the development of arthritis (4–9). Adoptive transfer of CIA to SCID mice or syngeneic T cell-depleted DBA/1 mice requires both T cells and anti-type II collagen (CII) Abs (10, 11). Taken together, these studies suggest an important role of MHC-restricted T cells in the development of arthritis.

Key functions of T cells are determined by the effector cytokines they produce in response to antigenic stimulation. Activated T cells can differentiate into effectors that produce predominantly either IFN-γ (type 1 response) or IL-4 and IL-5 (type 2 response) (12). The contribution of type 1 and type 2 responses in CIA is not completely understood. However, studies of cytokines at different stages of the disease revealed that the type 1 cytokine profile predominates at the induction and acute phases of the disease, whereas type 2 response is associated with the remission phase of the disease (13, 14), thus suggesting a pathogenic role of type 1 cytokines in CIA. In support of this hypothesis, a growing body of evidence shows that manipulation of the balance of cytokines produced by type 1/type 2 T cell subsets alters the disease outcomes (15–19).

Although T cells have long been recognized to be important in CIA, how specific molecular mechanisms of T cell activation influence the disease process is less clear. One family of transcription factors, the NF-κB/Rel family, has been increasingly implicated in immune regulation and inflammation (20, 21). NF-κB/Rel proteins are ubiquitous transcription factors that are activated in T lymphocytes after the engagement of the TCR, CD28, or other cell surface receptors. The prototypic form of NF-κB is a heterodimeric complex containing a trans-activating subunit in combination with either NF-κB1 (p50) or NF-κB2 (p52). The major trans-activating subunits of NF-κB that are induced during T cell activation are c-Rel and RelA (p65). In quiescent T cells, NF-κB is sequestered in the cytoplasm by a set of inhibitory molecules that includes IκBα. During normal T cell activation, IκBα undergoes signal-induced phosphorylation mediated by IκB kinases (IKKs) (22, 23), leading to subsequent degradation and translocation of NF-κB/Rel proteins into the nucleus to regulate gene transcription (24). Based largely on data from cell lines, many genes involved in T cell effector function are thought to be regulated by NF-κB/Rel proteins, including those that encode inflammatory cytokines and receptors, adhesion molecules, and chemokines (20). However, a number of such genes turn out not to be authentic NF-κB targets when primary cells are analyzed (25, 26). These findings raise unanswered questions about the role of NF-κB regulation in immune responses and the pathogenesis of autoimmune arthritis.

Departments of *Medicine/Rheumatology and Cell Biology and †Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232; and ‡Department of Pharmacology, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT 06877

Received for publication March 17, 1999. Accepted for publication May 20, 1999.

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1 This work was supported by grants from the American Heart Association (9730089N to J.C.), the Vanderbilt Cancer Center (ACS institutional research grant to I.C.), separate grants from Boehringer Ingelheim Pharmaceutical (to I.C. and M.B., respectively), the National Institutes of Health (AI-36997 to M.B.), the Vanderbilt Diabetes Research and Training Center (P60 DK20593 and the Mark Collie Pilot Project Fund to M.B.), and a Leukemia Society of America Scholar’s Award (to M.B.).

2 Address correspondence and reprint requests to Dr. Jin Chen, Medical Center North A4323, Vanderbilt University, Nashville, TN 37232. E-mail address: jin.chen@mcmail.vanderbilt.edu.

3 Abbreviations used in this paper: CIA, collagen-induced arthritis; CII, type II collagen; IKK, IκB kinase.
Elucidation of the in vivo roles of NF-κB/Rel in T cell activation in autoimmune diseases is complicated by the functional redundancy and embryonic lethality in mutant mice deficient for individual NF-κB/Rel subunits (21, 27–31). To circumvent these difficulties, a recent study showed that transgenic mice constitutively expressing a trans-dominant form of IkBα (IkBα(ΔN)) in their T lineage repress the activity of multiple NF-κB/Rel proteins (32). Of note, while high levels of transgene expression were maintained in mature T cells, these cells retained some responsiveness to activating signals delivered by TCR and CD28 co-stimulation. In this study, we investigated CIA in IkBα(ΔN) transgenic mice that had been backcrossed to the CIA-susceptible DBA/1 background. IkBα(ΔN) transgenic mice exhibited a delayed onset, lower incidence, and decreased severity of CIA. This inhibition of CIA is associated with a modest defect in proliferative response and a dramatic attenuation of IFN-γ production in response to CII. Our data show that inhibition of NF-κB/Rel activation impairs the development of an inflammatory autoimmune arthropathy in vivo, thereby providing an attractive target for therapeutic intervention.

Materials and Methods

Mice

DBA/1 mice were purchased from the The Jackson Laboratory (Bar Harbor, ME) and used at 8 wk of age. IkBα(ΔN) transgenic mice were derived from C57BL/6 and DBA/2 background (32). To introduce CIA susceptibility genes (the H-2d haplotype and other background genes), IkBα(ΔN) transgenic mice were crossed with DBA/1 for two generations (F1 N1). Mice were then screened for IkBα(ΔN) and H-2 by Southern blot analysis and PCR, respectively. The primer set 5'-ACCAAGGGACGACCGCAT3-3' and 5'-CCTCGTAGTTGTGTGCAC-3' was used to amplify 200 bp of product of the I-A β gene. The PCR products were separated on an agarose gel, transferred to a nylon membrane, and probed with an oligonucleotide primer specific for H-2d, H-2b, or H-2q genes. Primers 5'-ATACGGCTCGTGACCAGATA-3', 5'-ATACCGCTGGTGTGCAC3', and 5'-ATACGGCTCGTGACCAGATA3' were specific for H-2d, H-2b, or H-2q genes, respectively. IkBα(ΔN) transgenic mice homozygous for H-2d were then further backcrossed to DBA/1 for five additional generations (F5 N6).

Cell preparation

Single cell suspensions from thymus, spleen, and lymph node were prepared by crushing the organs in complete media (RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, and 0.1% penicillin-streptomycin), followed by hypotonic lysis of erythrocytes. Splenocytes were depleted of T cells by incubating with anti-Thy-1 Ab for 30 min at 4°C, followed by hypotonic lysis of erythrocytes. Splenocytes were depleted of T cells by incubating with anti-Thy-1 Ab for 30 min at 4°C, followed by hypotonic lysis of erythrocytes. Splenocytes were depleted of T and B cell populations using magnetic beads described previously (33). The paws were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Induction and assessment of CIA

Native bovine CII (Chondrex, Seattle, WA, and Dr. Marie Griffiths, University of Utah, Salt Lake City) was dissolved in 0.01 M acetic acid at 4°C overnight, and emulsified with an equal volume of CFA (Difco, Detroit, MI). Mice were injected intradermally at the base of the tail with 0.1 ml of emulsion containing 100 μg of CII; at 21 days after the primary immunization, mice were boosted with 0.1 ml of emulsion containing 100 μg of CII and IFA. Mice were analyzed every other day and monitored for signs of arthritis and date of disease onset in a blind fashion by two independent examiners. Clinical arthritis was assessed by using a scoring system, as follows: grade 0, no swelling; grade 1, paws with swelling in single joint; grade 2, paws with swelling in multiple joints; grade 3, severe swelling and joint rigidity. Each limb was graded, giving a maximum possible score of 12 per mouse. Data were analyzed using the Macintosh InStat software program. Group comparisons were performed using the χ2 test for disease incidence and unpaired, two-tailed Student’s t test for arithmetic scores.

Histology

Paws were removed postmortem, fixed in 4% paraformaldehyde, and decalcified in Immunocal solutions (Decal Chemical, Congers, NY), as described previously (33). The paws were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Proliferation assay

Mice were sacrificed 13 days after immunization. Draining lymph nodes (inguinal, paraaortic, and popliteal) were removed, and single cell suspensions were resuspended in DMEM supplemented with 2-ME and 1% autologous mouse serum. Lymph node cells (2 × 106/ml, 200 μl/well) were plated in 96-well round-bottom microtiters plates and stimulated with denatured bovine CII at 5 and 50 μg/ml. Cells were incubated at 37°C in 5% CO2 for 4 days, and 1 μCi/well of [3H]TdR was added in culture for the last 18 h. Cells were harvested and [3H]TdR uptake was measured using a beta scintillation counter.

Analysis of cytokines

Draining lymph node cells were removed 2 and 4 wk after immunization. Single cell suspensions were prepared and cultured in RPMI 1640 containing 10% FBS. The cells were cultured in 96-well plates for 72 h at 2 × 105/ml (200 μl/well) in medium alone, or with 5 or 50 μg/ml of heat-denatured bovine CII. Supernatants were collected and analyzed for IFN-γ and IL-4 by sandwich ELISA using Abs pairs (PharMingen, Sorrentino, CA), according to the manufacturer’s recommended procedures. The lower limits of sensitivity in the ELISA were 10 pg/ml (IL-4) and 20 pg/ml (IFN-γ), using mouse IFN-γ and IL-4 as standards (PharMingen).

Measurement of serum anti-CII Ab levels

Serum samples were collected from mice before immunization, 2, 4, and 6 wk after primary immunization for the detection of anti-CII IgG, IgG1, and IgG2a Ab levels. The level of serum Abs to CII was measured by ELISA either using a kit (Chondrex, Seattle, WA) or described briefly as follows. ELISA plates (Dynex Technologies, Chantilly, VA) were coated overnight at 4°C with 10 μg/ml native bovine CII in PBS. After washing with PBS containing 0.05% Tween-20 (PBST), nonspecific binding was blocked with PBS containing 2% skimmed milk for 1 h at room temperature. After washing three times, serum samples in serial dilutions from 1/100 to 1/105 were added and incubated for 2 h at room temperature. After three washes, alkaline phosphatase-conjugated goat anti-mouse IgG, IgG1, or IgG2a (Southern Biotechnology Associates, Birmingham, AL) were added and incubated at room temperature for 1 h, followed by six washes, and plates were developed using p-nitrophenol (Sigma) as substrate. The OD was measured using a microplate reader and Delta Soft 3 analytic software.

Cell reconstitution experiments

Wild-type CD8+ T cells were purified from pools of spleenocytes and lymph node cells isolated by positive selection using magnetic beads derived with Abs against mouse CD8a (PharMingen). A total of 5 × 108 CD8+ T cells in PBS were injected i.v. into each IkBα(ΔN) transgenic recipient mouse. Twenty-four hours after cell transfer, recipient transgenic and control mice were immunized with 100 μg bovine CII in CFA, boosted with 100 μg bovine CII in IFA at day 21, and monitored for signs of arthritis. Six weeks after primary immunization, spleenocytes and lymph node cells were isolated, stained with fluorochrome-conjugated mAbs against CD4 and CD8, and analyzed by flow cytometry.

Results

Specific expression of IkBα(ΔN) transgene in the T lineage of adult peripheral lymphoid organs

The important functions of NF-κB/Rel transcription factors in modulation of immune responses raise the possibility that they also play a key role in the pathogenesis of autoimmune arthritis. To

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investigate the role of NF-κB/Rel transcription factors in T cell activation during disease evolution in vivo, we utilized transgenic mice constitutively expressing an inhibitor of NF-κB (IkBα(ΔN)) in the T lineage (32). Because the transgene is under the control of the lck proximal promoter and the CD2 locus control region, we examined whether IkBα(ΔN) proteins were expressed in adult peripheral lymphoid organs and whether the expression of the transgene was restricted to the T lineage. The IkBα(ΔN) protein was readily detected in both lymph nodes and spleen from 4-mo-old transgenic mice (Fig. 1A, lanes 2, 3, 5, and 6) compared with nontransgenic littermates (Fig. 1A, lanes 1 and 4). To determine whether IkBα(ΔN) proteins were also made in cells other than T lineage, splenocytes were depleted of T cells by Thy-1-mediated complement lysis (lane 4). Cell lysates prepared from transgenic mice (Tg) and nontransgenic littermates (NTg) were immunoprecipitated (200 μg protein/lane) by anti-Flag mAb, and analyzed by Western blot, as described in A. Lanes 1 and 5, Thymocyte; lane 2, lymph node cells; lane 3, splenocyte; lane 4, T cell-depleted splenocyte.

To study CIA in IkBα(ΔN) mice, transgenic mice were backcrossed to the disease-susceptible DBA/1 (H-2d) background. After the first two backcrosses, the pups were genotyped for both transgene and H-2d gene, and H-2d homozygous transgenic mice were then selected for five additional backcrosses to DBA/1. The transgenic mice and nontransgenic littermates were then immunized with bovine CII in CFA, boosted with bovine CII in IFA, and monitored for the occurrence of clinical signs of arthritis. Four separate experiments were conducted and data are shown in Table I. The results from these four experiments were also pooled, and the incidence and the mean clinical scores of all animals in each group were calculated (Fig. 2). As shown in Table I and Fig. 2, IkBα(ΔN) provided protection against inflammatory arthritis, as measured by incidence, time of onset, and severity. The incidence in IkBα(ΔN) transgenic mice (39%, 9 of 23 mice) was significantly decreased as compared with nontransgenic littermates (100%, 22 of 22 mice; p < 0.01) (Table I and Fig. 2A). Severity of the disease was measured as the mean clinical scores reached by each group of mice (total clinical scores per group/numbers of animal in each group). As shown in Table I and Fig. 2B, arthritis scores were significantly decreased in IkBα(ΔN) mice (1.4 ± 1), compared with wild-type littermates (7.7 ± 1.1; p < 0.01). There

Table 1. CIA in IkBα(ΔN) transgenic mice

<table>
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<tr>
<td></td>
<td>Tg</td>
<td>9/23†</td>
<td>39</td>
<td>1.4 ± 1.0*†</td>
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* Mice were immunized with 100 μg of bovine CII in CFA on day 1 and boosted on day 21 with 100 μg of CII in IFA. Data are given as number of diseased mice for the incidence, as median of day of onset, and as the mean clinical scores reached by each group of mice (total clinical scores per group/numbers of animal in each group).

† Clinical severity of each joint was graded: grade 0, no swelling; grade 1, paws with swelling in single joint; grade 2, paws with swelling in multiple joints; grade 3, severe swelling and joint rigidity. Each paw was graded and the four scores were summed so that the maximum score per mouse was 12.

N/A: not applicable.

* Mean arthritic scores of four experiments; †, p < 0.01 vs nontransgenic littermates (χ² analysis); ‡, p < 0.01 vs nontransgenic littermates (Student’s t test).

Reduced incidence and severity of CIA in IkBα(ΔN) transgenic mice

![Graph showing decreased CIA incidence and severity in IkBα(ΔN) transgenic mice.](http://www.jimmunol.org/)
was also a delay in the development of disease in IκBα(ΔN) mice, because the median day of onset among diseased animals was day 30 for wild-type mice, and day 39 for those IκBα(ΔN) mice that became arthritic. Histology of joints was evaluated for mice sacrificed at 6–7 wk after immunization. Histological examination of affected joints (41 of 44 paws from 11 mice) from nontransgenic control mice showed typical arthritis, characterized by synoviocytes proliferation, pannus formation, and bone erosion (A). In contrast, most joints from IκBα(ΔN) mice (nine mice analyzed; 29 of 36 paws) showed either mild inflammation or no sign of inflammation. A representative section is shown here (B). Sections were excised from CII-immunized mice at 6–7 wk after immunization and stained with hematoxylin and eosin.

**FIGURE 3.** Histological examination of the joints from nontransgenic control mice and IκBα(ΔN) transgenic mice. Histological examination of affected joints (41 of 44 paws from 11 mice) from nontransgenic control mice showed typical arthritis, characterized by synoviocytes proliferation, pannus formation, and bone erosion (A). In contrast, most joints from IκBα(ΔN) mice (nine mice analyzed; 29 of 36 paws) showed either mild inflammation or no sign of inflammation. A representative section is shown here (B). Sections were excised from CII-immunized mice at 6–7 wk after immunization and stained with hematoxylin and eosin.

**FIGURE 4.** Proliferative response of lymph node cells to bovine CII. Draining lymph node cells from IκBα(ΔN) transgenic mice and nontransgenic littermates were isolated on day 14 after immunization, and proliferation was measured in medium alone or in response to bovine CII. Values represent the mean of five mice per group ± SEM, as analyzed in two separate experiments. Background counts for lymph node cells in transgenic mice and nontransgenic littermates are 1772 ± 277 and 9361 ± 2275, respectively. *, p < 0.01 vs nontransgenic littermates (Student’s t test).

**FIGURE 5.** Cytokine production in IκBα(ΔN) mice. Lymph node cell suspensions were prepared from draining lymph nodes from mice 2 and 4 wk after immunization with CII cultured for 72 h in the presence of 0, 5, or 50 μg/ml of CII, and supernatants were assayed for IFN-γ production by ELISA. Data are represented as the mean cytokine concentration ± SEM, as analyzed in three separate experiments. NTg, nontransgenic littermate controls (n = 11); Tg, IκBα(ΔN) transgenic mice (n = 11). *, p < 0.01 vs nontransgenic littermates (Student’s t test).
In sufficient to inhibit CIA

In the defects in IFN-γ-specific IgG2a Abs have been implicated in the pathogenesis of CIA. Anti-CII-specific IgG, IgG1, and IgG2a levels were measured by ELISA. Data are represented as the mean ± SEM using an arbitrary unit, as analyzed in four separate experiments. NTg, nontransgenic littermate controls (n = 14); Tg, IxBor(ΔN) transgenic mice (n = 14). *, p < 0.01 vs nontransgenic littermates; **, p < 0.05 vs nontransgenic littermates (Student’s t test).

Five million wild-type CD8+ cells were purified by positive selection using magnetic beads. This cell population alone could account for the protection against disease seen in IxBor(ΔN) transgenic mice. As shown in Fig. 5, CII-specific IgG2a levels were decreased substantially in transgenic mice compared with their nontransgenic littermates. The decrease in IgG2a levels in transgenic mice was most prominent after 2 wk immunization (p < 0.01), and persisted after 4 wk of immunization (p < 0.05). In contrast, no significant differences in IgG1 level were observed between IxBor(ΔN) mice and their control nontransgenic littermates (Fig. 6). These data, together with the observed decreases in IFN-γ production in transgenic mice, indicate that inhibition of NF-κB in vivo attenuates type 1 helper response.

Decreased CD8+ T cell population in transgenic mice is not sufficient to inhibit CIA

In IxBor(ΔN) mice, the level of CD8+ T cells is decreased in peripheral lymphoid organs (32). Because CD8+ T cells may play a role in initiation of CIA (34), in principle the decreased CD8+ T cell population alone could account for the protection against disease seen in IxBor(ΔN) mice. As an alternative, the attenuation of susceptibility might reflect changes in CD4+ T cell function as well. Therefore, we tested whether transfer of wild-type CD8+ T cells into IxBor(ΔN) mice could reverse IxBor(ΔN)-mediated inhibition of CIA. Transgenic mice and nontransgenic littermates backcrossed to DBA/1 for six generations were used for these reconstitution experiments. CD8+ cells from nontransgenic littermates were purified by positive selection using magnetic beads. Five million wild-type CD8+ cells were injected i.v. into each IxBor(ΔN) mouse. Three groups of mice, IxBor(ΔN) transgenic, nontransgenic littermate, and IxBor(ΔN) recipients of wild-type CD8+ cells, were immunized with CII 1 day after cell transfer and monitored for clinical signs of arthritis. To correlate the disease incidence and severity with the numbers of CD8+ T cells, we used flow cytometry to measure the level of CD8+ cells in lymph nodes and spleens of these three groups of mice 6 wk after immunization.

On the DBA/1 background, the level of CD8+ cells in IxBor(ΔN) mice is only about one-third of that in nontransgenic littermates (Fig. 7). In contrast, CD8+ cells in lymph node were restored to nearly normal in IxBor(ΔN) recipients of wild-type CD8+ cells. Similar results were obtained when CD8+ cells in spleen were enumerated (data not shown). Despite restoration of almost normal levels of wild-type CD8+ cells in IxBor(ΔN) transgenic mice, however, these mice still exhibited reduced disease incidence and severity (Table II). Taken together, our results suggest that neither defects of CD8+ population or function were sufficient to account for the disease-inhibition phenotype in transgenic mice.

Discussion

The role of T cells in CIA is well established, and the strong MHC association with susceptibility to rheumatoid arthritis suggests that T cell activation is important in the pathogenesis of inflammatory arthritis in humans. Although mice lacking mature lymphocytes can develop arthritic lesions after immunization with CII (35), a significant delay in disease onset and a substantial reduction of disease severity confirmed that lymphocytes play important roles in the disease. However, specific molecular mechanisms of T cell activation essential for normal disease pathogenesis are not clear. It has been reported that injection of liposomes containing NF-κB binding site DNA decoys inhibited streptococcal cell wall-induced arthritis in treated joints (36). However, these studies did not distinguish the roles of NF-κB in distinct cell populations. Thus, it remains unclear whether activation of NF-κB in T cells is important in arthritis. In this study, we show that inhibition of NF-κB signaling in the T cells of transgenic mice substantially attenuates the incidence and severity of CIA, demonstrating an important role of NF-κB in autoimmune disease in vivo.

NF-κB is regulated by a family of inhibitory molecules, including IκBα, IκBβ, IκBε, IκBγ, p100, and p105. It has been proposed that some members of the IκB family differentially activate Rel protein dimers that bind to distinct κB sites and regulate the expression of individual genes. Although the interaction specificity
of IκBα and IκBβ appears to be indistinguishable (they both bind to p50:RelA and p50:c-Rel heterodimer), there may be some subtle differences between IκBα and IκBβ, such as differential transcriptional regulation and their ability to act as a chaperone for c-Rel (37). Therefore, it is possible that individual actions of each inhibitor may act in concert to fine tune the complex regulation of NF-κB activation. Constitutive overexpression of IκBα(ΔN) in the transgenic mice may remove this fine tuning regulatory system, thereby leading to potent inhibition of NF-κB-mediated development of CIA.

How does inhibition of NF-κB activation in T cells lead to the inhibition of arthritis? In principle, at least three mechanisms can be envisaged. First, inhibition of NF-κB signaling may render T cells completely unresponsive, thereby blocking the development of CIA. Furthermore, inhibition of NF-κB/Rel signaling results in diminished numbers of CD8⁺ T cells, which might alone account for disease-inhibition phenotypes in IκBα(ΔN) transgenic mice. Finally, inhibition of CIA may be achieved by inhibition of IFN-γ production in IκBα(ΔN) transgenic mice.

Available data do not support the first hypothesis. Although proliferative responses and CII-specific Ab production are reduced in the IκBα(ΔN) transgenic mice, IκBα(ΔN) T cells do proliferate in response to CII challenge, and IκBα(ΔN) mice produce significant amounts of CII-specific IgG (especially at 4 and 6 wk postimmunization). Furthermore, costimulation of primary T cells with anti-CD3 and anti-CD28 restored the production of IL-2 close to normal level in transgenic mice (38). These results, together with the fact that full induction of Ag-specific IgG1 are not reduced in IκBα(ΔN) transgenic mice, suggest that IκBα(ΔN) T cells are capable of providing some helper effector functions.

We consider that the second hypothesis is also unlikely. The role of CD8⁺ cells in CIA is not well defined. A recent study showed that CD8-deficient mice have a moderate decrease in CIA incidence without affecting disease severity, and increased disease incidence with repeat immunization (34). Our results are inconsistent with a mechanism in which inhibition of CIA was due solely to a decrease in CD8⁺ population or defective CD8 function, because the IκBα(ΔN) transgenic mice exhibit more profound decrease in disease severity as well as disease incidence compared with CD8-deficient mice. In addition, our data showed that transfer of wild-type CD8⁺ cells into transgenic recipients restored the CD8⁺ compartment to near normal level, but failed to rescue disease susceptibility (Fig. 7). Taken together, our results suggest that neither defects of CD8⁺ population nor function were sufficient to account for the disease-inhibition phenotype in transgenic mice.

We favor the interpretation that inhibition of CIA is achieved by inhibition of IFN-γ-producing cells in IκBα(ΔN) transgenic mice. First, transgenic mice produced substantially decreased amounts of IFN-γ compared with controls when draining lymph node cells were restimulated with CII in vitro. Furthermore, the cytokine data are supported by a change in the ratio of IgG1 to IgG2a isotypes of anti-CII Abs in serum, with decreased IgG2a and normal level of IgG1. Consistent with these observations, IκBα(ΔN) transgenic mice exhibited attenuation of delayed-type hypersensitivity response and reduced IgG2a despite substantial eosinophil recruitment and normal level of IgE in an allergic lung disease model (M. Aronica and M. Boothby, personal communication). Taken together, these data suggest that development of IFN-γ responses in vivo is dependent on NF-κB/Rel signaling. This decrease in type 1 T cell effector function could be due to the direct regulation of the IFN-γ expression by NF-κB (39), or through enhanced apoptosis of effector Th1 cells (40).

Although it is generally thought that CIA is a predominantly Th1 disease, the exact role of IFN-γ in CIA is more controversial because disease-promoting as well as disease-limiting effects have been discerned (41-44). Mechanistically, IFN-γ can promote disease through enhancing Ag presentation, by augmenting expression of MHC class II and cell adhesion molecules, or promoting Th1 cell differentiation and activation of macrophages (45). On the other hand, IFN-γ has other immune regulatory roles (46) such that inactivation of IFN-γ receptor accelerates CIA (43, 44). The outcome of disease after direct interference with IFN-γ signaling probably reflects the balance of these two opposing roles of IFN-γ in vivo. Our data are consistent with the disease-promoting role of IFN-γ in CIA. Because IFN-γ production is not completely abrogated in IκBα(ΔN) transgenic mice, the outcome of balancing two opposing effects of IFN-γ level in vivo appears to be the inhibition of CIA in IκBα(ΔN) transgenic mice.

Rheumatoid arthritis is a leading cause of long-term disability in the United States. Current therapeutic strategies have limitations, and additional targets and approaches are needed for treatment of rheumatoid arthritis patients. NF-κB/Rel transcription factor appears to be a good target for therapeutic intervention. First, NF-κB is activated in human RA synovium (47, 48). Furthermore, intra-articular liposomal delivery of a similar IκBα mutant suppressed recurrent streptococcal cell wall-induced arthritis in rats (36). Although the above studies did not distinguish the roles of NF-κB in distinct cell populations, our study suggests that transcription factor NF-κB is a suitable target for modulating inflammatory T cell function in vivo. With the discovery and cloning of NF-κB-activating kinases such as IKK-1 and IKK-2, it has become possible to design small molecules that modify the activities of these kinases, but few such agents will be able to achieve 100% inhibition without unacceptable side effects. The incomplete inhibition of NF-κB in IκBα(ΔN) mice thus provides an important model for dissecting the contribution of NF-κB signaling to T cell activation, cytokine
production, and apoptosis. Our findings in collagen-induced arthritis suggest that even this partial inhibition can attenuate a T cell-dependent inflammatory disease in which IFN-γ production is pathophysiologically important.

Acknowledgments
We thank Drs. James (Tom) W. Thomas and Gerry Miller for their support and helpful discussions. We thank Dr. Mark Aronica for generously sharing data before publication; Drs. Gerry Miller, James (Tom) W. Thomas, Mark Aronica, and Roy Fava for critical reading of manuscript; Matt McReynolds and Anita Ellerby-Brown for technical assistance; Drs. Robert A. Reife and Kuniaki Terada (Contrex, Seattle, WA) for consultation; and D. MacFarland (Howard Hughes Medical Institute Flow Cytometry Core) for assistance with flow cytometry.

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