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Redirection of T Cell Effector Function In Vivo and Enhanced Collagen-Induced Arthritis Mediated by an IL-2R β /IL-4R α Chimeric Cytokine Receptor Transgene

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Redirection of T Cell Effector Function In Vivo and Enhanced Collagen-Induced Arthritis Mediated by an IL-2R β /IL-4R α Chimeric Cytokine Receptor Transgene¹

Ying Chen,* Ed Rosloniec,[†] Mehmet I. Goral,[‡] Mark Boothby,[§] and Jin Chen^{2*}

Chronic inflammatory autoimmune diseases such as diabetes, experimental autoimmune encephalomyelitis, and collagen-induced arthritis (CIA) are associated with type 1 (Th1, Tc1) T cell-dependent responses against autoantigens. Immune deviation toward type 2 (Th2, Tc2) response has been proposed as a potential means of gene therapy or immunomodulation to treat autoimmune diseases based on evidence that type 2 cytokines can prevent or alleviate these conditions. In this report we assessed the effects of elevated type 2 responses on CIA using transgenic mice expressing an IL-2R β /IL-4R α chimeric cytokine receptor transgene specifically in T cells. In response to IL-2 binding, this chimeric receptor transduces IL-4-specific signals and dramatically enhances type 2 responses. In contrast to published reports of Th2-mediated protection, CIA was exacerbated in IL-2R β /IL-4R α chimeric receptor transgenic mice, with increased disease incidence, severity, and earlier disease onset. The aggravated disease in transgenic mice was associated with an increase in type 2 cytokines (IL-4, IL-5, IL-10) and an increase in collagen-specific IgG1 levels. However, IFN- γ production is not affected significantly in the induction phase of the disease. There is also an extensive eosinophilic infiltration in the arthritic joints of the transgenic animal, suggesting a direct contribution of type 2 response to joint inflammation. Taken together, our findings provide novel evidence that enhancement of a polyclonal type 2 response in immunocompetent hosts may exacerbate an autoimmune disease such as CIA, rather than serving a protective role. This finding raises significant caution with regard to the potential use of therapeutic approaches based on immune deviation toward type 2 responses. *The Journal of Immunology*, 2001, 166: 4163–4169.

Effector T cells play a critical role in controlling pathological immune responses, including infections and allergic and autoimmune disorders. According to the pattern of cytokine production, effector T cells can be classified into functionally distinct subsets that include type 1 (Th1 and Tc1) and type 2 (Th2 and Tc2) cells (1, 2). Type 1 cells produce IFN- γ , TNF- β , IL-2, and low levels of IL-10, whereas type 2 cells produce IL-4, IL-5, IL-13, and high levels of IL-10. Type 1 cells are critical for cell-mediated immunity and inflammatory responses such as diabetes and experimental autoimmune encephalomyelitis (EAE).³ In contrast, type 2 cells mediate humoral and mucosal immunity and allergy, including the activation of eosinophils and mast cells and the production of IgE. Collagen-induced arthritis (CIA) is an animal model of autoimmune disease that has been extensively used to elucidate pathogenic mechanisms relevant to human rheumatoid

arthritis (3, 4). The contribution of type 1/type 2 responses in CIA is not completely understood, because both humoral and cell-mediated immune mechanisms are required for the development of full-blown disease. Mauri et al. reported that a type 1 cytokine profile predominates in the induction and acute phases of the disease, while type 2 responses are associated with the remission phase of disease (5). However, there is a transient increase in IL-10 during the induction phase of the disease. Doncarli et al. analyzed the frequency of Th1- or Th2-like type II collagen (CII)-specific T cell clones (6) isolated from different stages of CIA. Among lines generated 8 days after immunization, 60% were Th0-like, 25% were Th1-like, and 15% were Th2-like compared with 33% Th0-like, 11% Th1, and 56% Th2 by day 25 after immunization before clinical arthritis. Although these data are consistent with a pathogenic role of Th1 cells in CIA, they do not explain the role or potential contribution of Th2 cells and related cytokines to the development of CIA.

Altering the balance of cytokines produced by effector subsets of T cells has been under investigation as a therapeutic approach to alter the outcome of arthritis. For example, the administration of both IL-4 and IL-10 to arthritic animals ameliorates arthritis, whereas IL-10-neutralizing Abs accelerate disease onset and increase disease severity (7–9). In contrast, the incidence and severity of arthritis are decreased in IL-12 and IFN regulatory factor-1-deficient mutant mice (10, 11). Although these data support CIA as a type 1 disease, this straightforward model is complicated by the contradictory roles of IFN- γ and IL-12: disease promoting as well as disease limiting (12–17). Some of these differences may arise because the experimental and genetic manipulations used typically have affected APCs, costimulatory function, and nonhemopoietic cells in addition to the T cell compartment.

To investigate the in vivo regulation of the balance of type 1 and type 2 cells, we generated a transgenic mouse line in which an

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³ Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; CIA, collagen-induced arthritis; CII, type II collagen.

IL-2R β /IL-4R α (IL-2R/IL-4R) chimeric cytokine receptor is expressed specifically in the T lineage but not in APCs or other tissue (18). This chimeric receptor transduces signals characteristic of IL-4 in response to IL-2 binding. Although the transgenic mice exhibit normal development of the T lineage, peripheral T cells from these transgenic mice exhibited enhanced type 2 responses. This influence on the development of effector T cells was sufficient to overcome the resistance of C57BL/6 mice to OVA-induced allergic airway disease, a disease mediated by type 2 T cells. Thus, in chimeric receptor transgenic mice, the presence of IL-2 upon antigenic challenge appears to favor differentiation of naive T cells into type 2 effector cells.

Because type 2 cytokines are considered to be beneficial in CIA, we hypothesized that expression of IL-2R/IL-4R chimeric receptor in T cells in our transgenic mice should protect animals from disease. Unexpectedly, CIA was exacerbated in chimeric receptor transgenic mice. Here we report that disease exacerbation is associated with selective enhancement of type 2 response without significantly affecting IFN- γ level at the induction phase of the disease. Moreover, an extensive eosinophil infiltration was present in the arthritic joints. Taken together, these results suggest that augmentation of type 2 response in IL-2R/IL-4R transgenic mice can be pathogenic, a result with significant implications with regard to the potential use of immune deviation as a therapeutic approach.

Materials and Methods

Mice

DBA/1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used at 6–7 wk of age. IL-2R β /IL-4R α transgenic mice were derived from C57BL/6 and DBA/2 background. To introduce CIA susceptibility genes (the H-2^q haplotype and other background genes), IL-2R β /IL-4R α transgenic mice were crossed with DBA/1 for two generations (F₁N₁). Mice were then screened for IL-2R β /IL-4R α transgene by Southern blot analysis and PCR. The primer set 5'-GAGCCAGCCCCTGACCTTTC-3' and 5'-GGGAAGTCTGCTTCTTCTT-3' was used to amplify 350 bp of product of the IL-2R β /IL-4R α gene. The H-2 haplotype was also identified by PCR. The primer set 5'-ACCAACGGGACGCAGCGCAT-3' and 5'-CCTCGTAGTTGTGTGTCAC-3' was used to amplify the 200 bp of product of the I-A β gene. The PCR products were then probed with oligonucleotides specific for H-2^q, H-2^b, or H-2^d genes (5'-ATACGATCTGTGAACAGATA-3', 5'-ATACGATATGTGACCAGATA-3', and 5'-ATACGGCTCGTGACCAGATA-3' were specific for H-2^q, H-2^b, and H-2^d genes, respectively). IL-2R β /IL-4R α transgenic mice homozygous for H-2^q were then further backcrossed to DBA/1 for four or five additional generations (F₁N₅-F₁N₆). Collagen-specific DBA1 V β 8.3 tg mice were developed by Ed Rosloniec (19). Primer set 5'-CTCTTCTA GAACACATGGAGG-3' and 5'-GACAGACAGCTTGGTTCCATG-3' was used to amplify 346 bp of the product of the V β 8.3 gene.

Induction and assessment of CIA

Native bovine CII (Chondrex, Seattle, WA) was dissolved at 2 mg/ml in 0.01 M acetic acid at 4°C overnight and emulsified with an equal volume of CFA (Difco, Detroit, MI). CIA was induced by intradermal injection at the base of the tail with 100 μ l of emulsion containing 50 μ g of CII. Starting 21 days after immunization, 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 digit; grade 2, paws with swelling in multiple digits; grade 3, severe swelling and joint rigidity. Each limb was graded, giving a maximum possible score of 12/mouse. Data were analyzed using the Macintosh InStat software program. Group comparisons were performed using the χ^2 test for disease incidence and unpaired, two-tailed Student's *t* test for arthritic scores.

Histology

Paws were removed postmortem, fixed in 4% paraformaldehyde, and decalcified in Immunocal solutions (Decal Chemical, Congers, NY). The paws were then embedded in paraffin and sectioned. Eosinophil-specific Luna staining was used to demonstrate tissue eosinophilia. To compare quantitative measures of the tissue eosinophilia in transgenic mice (*n* = 8)

and nontransgenic control littermates (*n* = 8), eosinophils were counted in 10 fields/cross-section of each joint in a blind fashion.

Proliferation assay

Mice were sacrificed on day 10 after immunization. Draining lymph nodes (inguinal and para-aortic) were excised, and single-cell suspensions were resuspended in serum-free HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with L-glutamine, 2-ME, and antibiotics. Lymph node cells (4×10^5 /well) were plated in triplicate in 96-well flat-bottom microtiter plates in medium alone or in the presence of denatured bovine CII at 5 and 50 μ g/ml, or CII peptides at 3.3 and 33 μ g/ml. Bovine CII synthetic peptide (ATGPLGPKGQTGEBGIAGFKGEQGPK) was a gift from D. D. Brand (University of Tennessee, Memphis, TN). Cells were incubated at 37°C in 5% CO₂ for 4 days, and 1 μ Ci/well of [³H]TdR was added in culture for the last 18 h. Cells were harvested, and [³H]TdR incorporation per well was measured using a beta scintillation counter.

Cytokine assays

Draining lymph nodes were removed 2 and 7 wk after immunization. Single-cell suspensions were prepared and cultured in RPMI 1640 containing 10% FBS. The cells were cultured in 96-well round-bottom plates for 72 h at 2×10^6 cells/ml (200 μ l/well) for 72 h in medium alone or with 5 or 50 μ g/ml of heat-denatured bovine CII. Supernatants were harvested and analyzed for IL-4, IL-5, IL-10, and IFN- γ by sandwich ELISA using Ab pairs (PharMingen, Sorrentino, CA), according to the manufacturer's recommended procedures.

Measurement of serum anti-CII Ab levels

Serum samples were collected before immunization and 2, 4, and 6 wk after immunization for the detection of CII-specific IgG, IgG1, and IgG2a levels. The levels of serum anti-CII Abs were measured by ELISA as previously described (20). In brief, ELISA plates (Dynex, Chantilly, VA) were coated overnight at 4°C with 10 μ g/ml native bovine CII in 0.1 M NaHCO₃ (pH 9.6). After washing with PBS containing 0.05% Tween 20, nonspecific binding was blocked by PBS containing 1% BSA for 1 h at room temperature. All serum samples were tested in serial dilutions from 1/100 to 1/10⁵. After overnight incubation at 4°C, the samples were washed with PBS containing 0.05% Tween 20; incubated with alkaline phosphatase-conjugated goat anti-mouse IgG, IgG1, and IgG2a (Southern Biotechnology Associates, Birmingham, AL) at room temperature for 1 h, followed by six washes; and plates were developed using *p*-nitrophenyl phosphate (Sigma, St. Louis, MO) as substrate. The OD was measured at 420 nm using a microplate reader and Δ 3 software. A standard serum, i.e., mixture of sera from wild-type arthritic mice, was added at each plate in serial dilutions, and a standard curve was generated to design arbitrary units of total IgG, IgG1, and IgG2a anti-CII Abs.

Results

Exacerbated CIA in IL-2R/4R transgenic mice

To study CIA in IL-2R/4R chimeric receptor mice, transgenic mice were backcrossed to the disease-susceptible DBA/1 (H-2^q) background. After the first two crosses, the pups were genotyped for both the transgene and the H-2^q gene. H-2^{q/q} homozygous transgenic mice were then selected for four additional backcrosses to DBA/1. Because our original hypothesis was that T cell-specific expression of IL-2R/IL-4R chimeric receptor would serve a protective role in CIA, we initially induced disease by immunizing transgenic mice and control littermates with 100 μ g of bovine CII in CFA and boosted with 100 μ g of bovine CII in IFA 3 wk later. To our surprise, transgenic mice exhibited accelerated disease onset, although disease incidence and severity were not altered under this immunization protocol. Disease developed on day 21 after primary immunization in transgenic mice compared with day 31 in control littermates (data not shown). Under this immunization protocol DBA/1 mice develop severe arthritis with an incidence approaching 100%, thus making it difficult to assess the potentially increased severity of disease in the transgenic mice as suggested by the earlier onset. Therefore, we altered our immunization protocol to a single injection of 50 μ g of CII in CFA. The results of three separate experiments using this less intensive induction regimen are summarized in Table I. The incidence and arthritis index

Table I. CIA in IL2R/4R chimeric receptor transgenic mice^a

Expt.	Mice	Incidence	Day of Onset	Arthritis Index ^b
1	NTg	1/6	42	10
	Tg	2/6	28	10.5
2	NTg	1/7	44	5
	Tg	2/5	23	8.3
3	NTg	3/7	33	5.7
	Tg	4/5	24	10.75
Total	NTg	5/20	42	6.4 ± 1.3*
	Tg	8/16 [§]	24	9.9 ± 0.9* [¶]

^a Mice were immunized with a single dose of 50 μg of bovine CII in CFA. Data are given as number of diseased mice for the incidence, as median of day of onset, and as arthritis index (total clinical scores per group/numbers of arthritic animals in each group). Tg, IL-2R/4R chimeric receptor transgenic mice; NTg, nontransgenic littermates.

^b 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.

*, Arthritic index of three experiments; §, *p* < 0.05 vs nontransgenic littermates (χ^2 analysis); ¶, *p* = 0.05 vs nontransgenic littermates (Student's *t* test).

of animals in each group were calculated from pooled data of these experiments (Fig. 1). Under this new immunization protocol, it was clear that CIA is exacerbated in transgenic mice, as indicated by an increase in disease incidence and severity and earlier disease onset. As shown in Table I and Fig. 1, the incidence in transgenic mice (50%, 8 of 16 mice) was significantly increased compared with that in nontransgenic littermates (25%, 5 of 20 mice; *p* < 0.05). The severity of the disease was measured as an arthritis index (total clinical scores per group/numbers of arthritic animals in each group). The arthritis index was also significantly increased in IL-2R/4R transgenic mice (9.9 ± 0.9; *p* = 0.05), compared with wild-type littermates (6.4 ± 1.3). In addition, there was an acceleration in disease onset in transgenic mice, because the median day of onset among diseased mice was day 24 for IL-2R/4R transgenic mice but day 42 for arthritic wild-type mice. Taken together, these results clearly demonstrate that expression of IL-2R/4R chimeric receptor in T cells in transgenic mice exacerbated arthritis.

Normal Ag-specific proliferative response in IL-2R/4R transgenic mice

To investigate the mechanism by which altered T cell function in these transgenic mice leads to exacerbation of the disease, we first tested Ag-specific proliferative responses of lymph node cells from transgenic mice and their nontransgenic littermates. Transgenic and control mice were immunized with bovine CII, and draining lymph node cells were isolated and rechallenged with 50 μg/ml of CII or synthetic peptide in culture. As shown in Fig. 2A, there was no significant difference between proliferation of IL-2R/4R transgenic lymph node cells and control cells from nontransgenic lit-

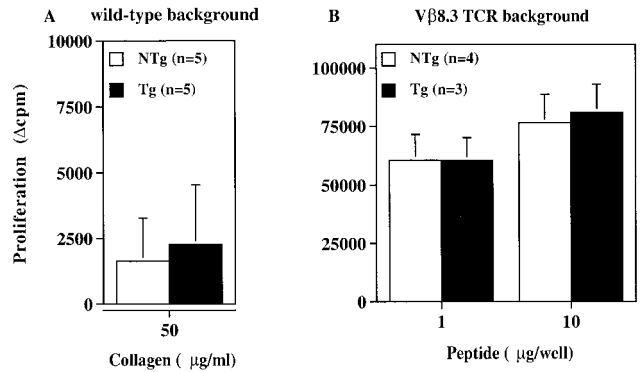


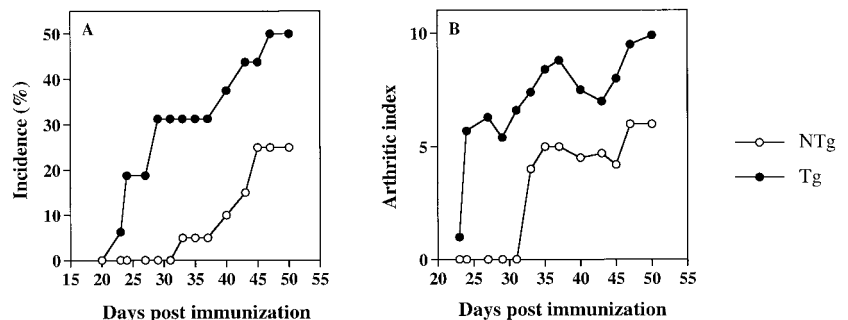
FIGURE 2. Proliferative response of lymph node cells to bovine CII. Draining lymph node cells from IL-2R/IL-4R transgenic mice and nontransgenic littermates on wild-type (A) and CII-specific TCR $\nu\beta 8.3$ transgenic background (B) were isolated on day 10 after immunization, and proliferation was measured in medium alone or in response to bovine CII or CII peptides. Values represent the mean per group ± SEM, as analyzed in two separate experiments. Background counts (counts per minute) for lymph node cells in chimeric receptor transgenic mice and nontransgenic littermates are 7,085 ± 1,305 and 5,874 ± 1,740 on wild-type background (A) and 73,478 ± 25,651 and 84,893 ± 34,247 on CII-specific TCRV $\beta 8.3$ background (B).

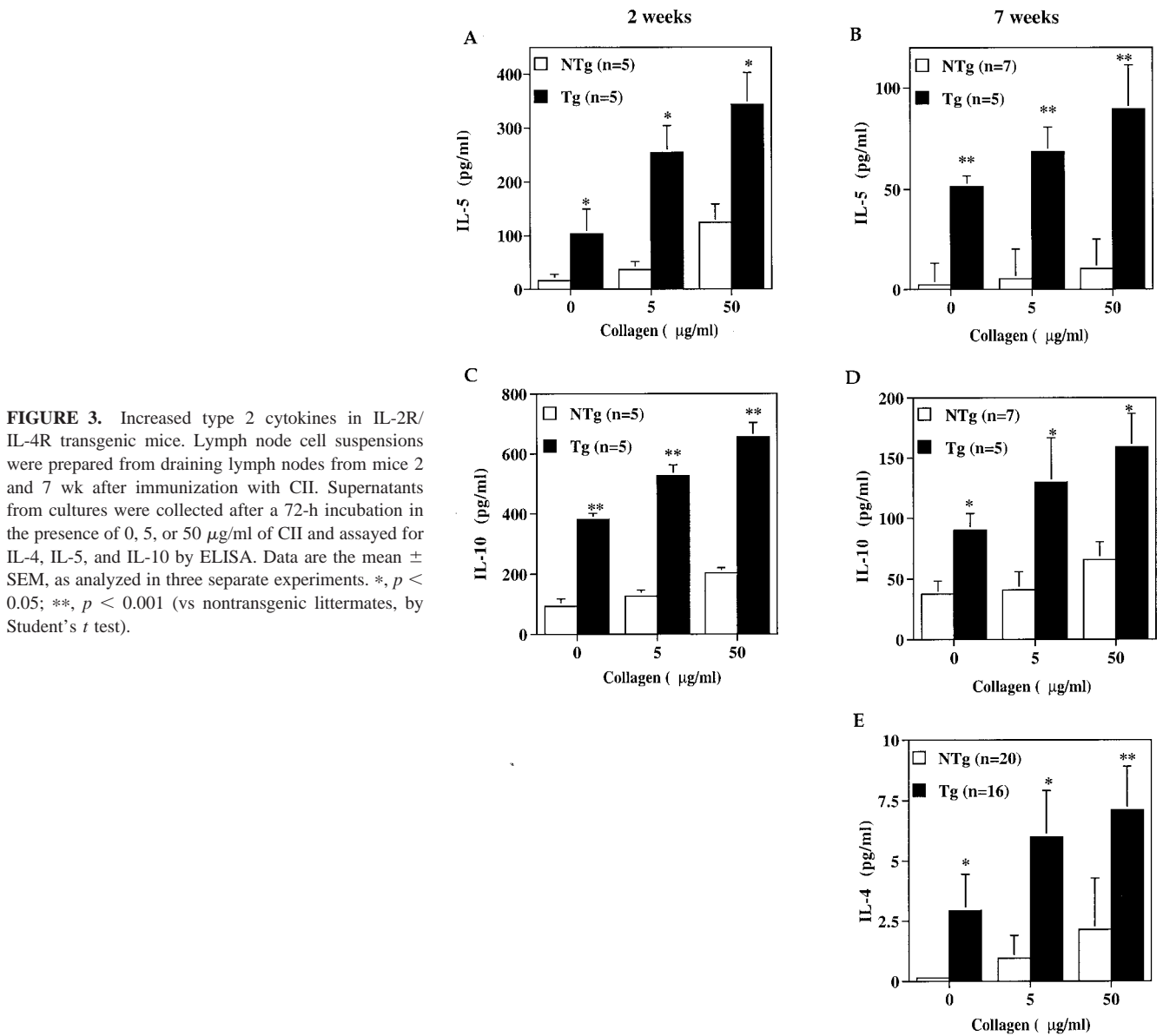
termates (at 50 μg/ml of CII). We also measured the proliferative response of T cells expressing a CII-specific V $\beta 8.3$ -TCR transgene (19) when the chimeric receptor was present or absent. The results (Fig. 2B) showed that lymph node cells from both control and chimeric receptor transgenic mice responded strongly to CII peptide in a dose-dependent manner, but there was no significant difference between transgenic mice and control littermates. These data suggest that the exacerbation of arthritis in IL-2R/4R transgenic mice was not due to enhanced T cell proliferation in response to CII challenge in IL-2R/4R transgenic mice.

Selectively enhanced type 2 cytokine response in IL-2R/4R transgenic mice

Given that the chimeric receptor amplified IL-4-signaling events during in vitro differentiation (18), we determined the effect of the chimeric receptor on the production of IL-4, IL-5, and IL-10 in response to collagen stimulation in vivo. As shown in Fig. 3, both IL-5 and IL-10 levels were drastically increased in the IL-2R/4R transgenic mice compared with nontransgenic littermates (Fig. 3, A–D). Production of IL-4 was below the limit of detection at 2 wk postimmunization, but the level of IL-4 was significantly increased at 7 wk postimmunization (Fig. 3E). On a CII-specific V $\beta 8.3$ TCR transgenic background, a dramatic increase in IL-4 level in chimeric receptor transgenic animals was detected as early as 10 days postimmunization (Fig. 5B). Although these results demonstrate that T cell-specific expression of chimeric receptor enhances type 2 help, IFN- γ

FIGURE 1. Increased CIA incidence and severity of arthritis in IL-2R/4R chimeric receptor transgenic mice. Data are shown for the incidence of arthritis (A) and severity of clinical signs (B) in IL-2R/4R transgenic mice (●) and nontransgenic littermates (○) after immunization with bovine CII as described in Table I. Results are pooled from three separate experiments (shown in Table I) and expressed as a percentage of the value in arthritic mice (A) and as the arthritis index of all mice in each group on a given day during the course of CIA (B).





production was not significantly affected in the induction phase of the disease in the transgenic mice (Figs. 4A and 5A).

Increased Ag-specific IgG1 Ab level in IL-2R/4R transgenic mice

Because development of CII-specific Abs requires T cell help, we measured the effect of the transgene on type 2 help in vivo by

measuring CII-specific IgG, IgG1, and IgG2a production. Mice were immunized with CII in CFA, CII-specific Ab levels were determined in preimmune sera, and samples were collected at 2, 4, and 6 wk after immunization. CII-specific IgG, IgG1, and IgG2a were undetectable before immunization, and there was no significant difference in the IgG2a level between transgenic and control mice after immunization. In contrast, the CII-specific IgG1 and

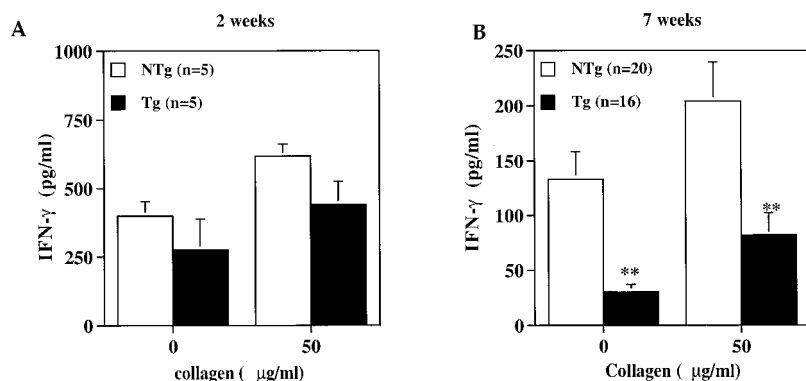
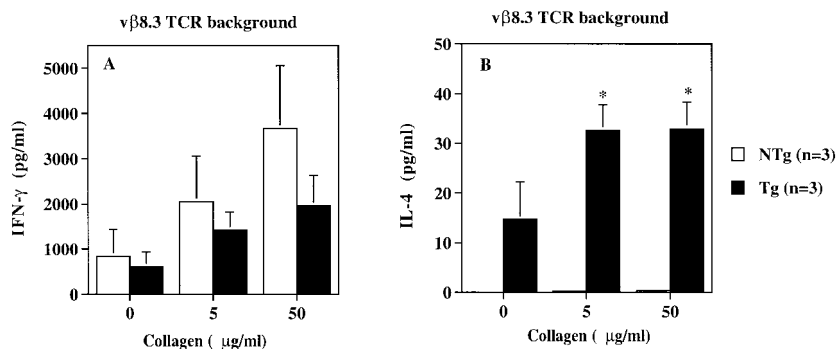


FIGURE 4. Diminished IFN-γ production in IL-2R/IL-4R transgenic mice. Lymph node cell suspensions were prepared from draining lymph nodes obtained from mice 2 and 7 wk after immunization with CII. Supernatants from cultures were collected after a 72-h incubation in the presence of 0, 5, or 50 µg/ml of CII and assayed for IFN-γ by ELISA. Data are the mean ± SEM, as analyzed in three separate experiments. *, $p < 0.05$; **, $p < 0.001$ (vs nontransgenic littermates, by Student's *t* test).

FIGURE 5. Cytokine production in chimeric receptor transgenic mice on CII-specific TCR V β 8.3 background. Lymph node cell suspensions were prepared from draining lymph nodes from mice 10 days postimmunization with CII. Supernatants from cultures were collected after a 72-h incubation in the presence of 0, 5, and 50 μ g/ml of CII and assayed for IL-4 and IFN- γ levels by ELISA. *, $p < 0.05$ (vs nontransgenic littermates, by Student's t test).



IgG levels were significantly increased in chimeric receptor transgenic mice compared with their nontransgenic littermate controls (Fig. 6) 2 and 4 wk after immunization. This elevated IgG1 production persisted 6 wk after immunization (Fig. 6), consistent with our cytokine data, which suggest that T cell-specific expression of the transgene enhances type 2 help in vivo in CIA. Taken together, we conclude from these studies that the accelerated arthritis of chimeric receptor transgenic mice is not attributable to type 1 T cells. Instead, type 2 help was increased along with a significant increase in total CII-specific IgG.

Recruitment of eosinophils in arthritic joints in IL-2R/4R transgenic mice

A hallmark of inflammatory arthritis such as RA and CIA is the presence of pannus, proliferating synovial membranes that form interdigitating folds. The formation of active inflamed pannus results in the destruction of cartilage and bone. Recent reports showed that local IL-4 gene therapy could protect cartilage and bone from erosion despite severe inflammation (21). To determine the effect of enhanced type 2 response in our transgenic mice on cartilage and bone, we examined joint histology in arthritic mice. When paws with equivalent degrees of clinical inflammation were examined, there were no significant differences in cellular infiltration, pannus formation, and cartilage and bone erosion between transgenic mice and nontransgenic littermates (data not shown). However, there was extensive accumulation of eosinophils in IL-2R/4R transgenic mice, while eosinophils were rarely detected in the joints of nontransgenic littermates with the same clinical score (Fig. 7, A and B). The majority of the eosinophils accumulated in

areas adjacent to blood vessels in the dermis. When the degree of eosinophilia was quantitated (Fig. 7C), there was an increase in the numbers of eosinophils with the increase in disease severity, with most abundant eosinophils found in the joints of transgenic mice with grade 3 disease. In contrast, eosinophils were barely detectable in nontransgenic littermates regardless of the severity of the disease. These data are consistent with high level IL-4 and IL-5 production in transgenic mice 7 wk postimmunization (Fig. 3, E and B). Taken together, our results suggest that increased production of IL-4 and IL-5 by type 2 T cells leads to eosinophil recruitment and activation in the joint, exacerbating inflammation in chimeric receptor transgenic mice.

Discussion

Although there has been debate as to whether CIA is a Th1 disease, Th2 cytokines are frequently considered to be beneficial for the disease. Thus, treatment of arthritic animals with IL-4 or IL-10 ameliorates the disease, whereas neutralizing anti-IL-4 and anti-IL-10 Abs accelerate disease onset and increase disease severity. Indeed, such data have suggested that gene therapy designed to enhance Th2 responses might prevent or ameliorate autoimmune diseases such as arthritis. Here we have used a transgenic mouse line that expresses an IL-2R/4R chimeric cytokine receptor in the T lineage so that it transduces IL-4-specific signals in response to IL-2 binding and dramatically enhances type 2 responses (18). Unlike unregulated Th2 responses in IL-4 transgenic or Bcl-6 knockout mice (22–24), which induce defects in multiple organs, chimeric receptor transgenic mice express the transgene specifically in T cells and exhibit normal development of the T lineage,

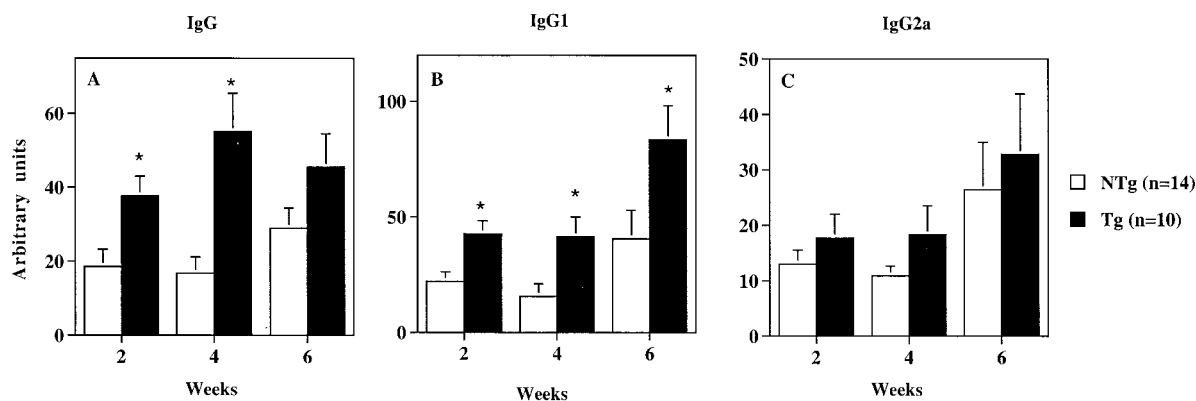


FIGURE 6. Increased CII-specific IgG and IgG1 responses in IL-2R/IL-4R transgenic mice. Serum samples were collected 2, 4, and 6 wk after immunization with CII. Anti-CII-specific IgG, IgG1, and IgG2a levels were measured by ELISA. Data are the mean \pm SEM using an arbitrary unit, as analyzed in three separate experiments. NTg, Nontransgenic mice; Tg, IL-2R/IL-4R transgenic mice. *, $p < 0.05$ (vs nontransgenic littermates, by Student's t test).

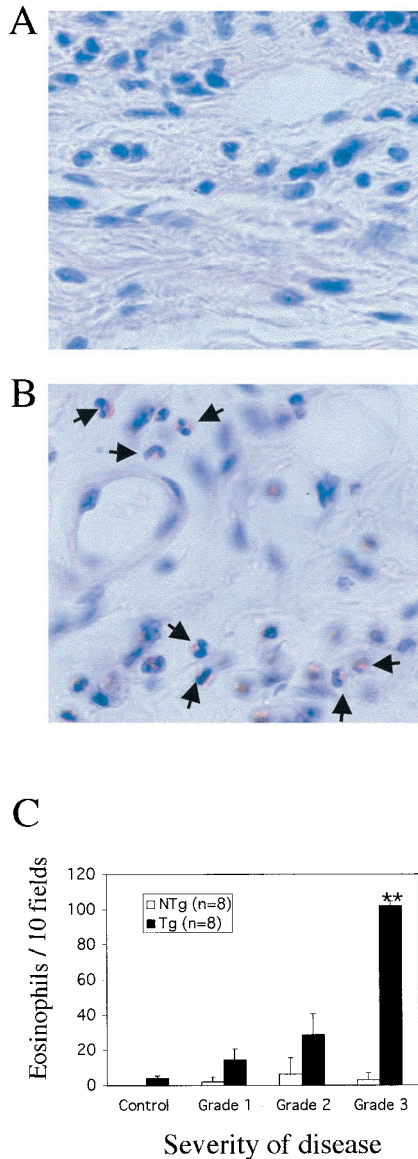


FIGURE 7. Eosinophil infiltration in joint of IL-2R/IL-4R transgenic mice. Nontransgenic and IL-2R/IL-4R transgenic mice were immunized with CII as described in *Materials and Methods*. Seven weeks after immunization joint eosinophilia was evaluated by staining joint sections with Luna's stain. Representative joint sections (original magnification, $\times 100$) from nontransgenic (A) and transgenic (B) CII-sensitized mice are shown in *upper* and *lower panels*, respectively. Arrows indicate eosinophils. C, Eosinophilia in transgenic mice (Tg; $n = 8$) and nontransgenic control littermates (NTg; $n = 8$) were quantitated by counting in 10 fields/cross-section of each joint in a blind fashion. Disease severity was graded as described in Table I.

and their immune responses are regulated specifically by antigenic challenge. In contrast to the results with neutralizing Abs and administration of cytokines, CIA is exacerbated in chimeric receptor transgenic mice.

One interpretation of our results might have been that elevated IL-4 production somehow also promotes a type 1 response in vivo in transgenic mice. A recent report showed that IL-4 enhances IL-12 production by dendritic cells and thereby promotes a Th1 response (25). Moreover, IL-4 has been shown to enhance Th1 cell development in vitro in the presence of TGF- β (26). However, this hypothesis is rendered unlikely by the finding that IFN- γ production induced by collagen in transgenic mice either was not signif-

icantly altered during the induction phase of the disease or was diminished in later phases of the disease. Our data suggest that T cell-specific expression of chimeric receptor leads to selective enhancement of Th2 cell development in vivo. An alternative interpretation is that the more severe disease phenotype of transgenic mice is directly caused by increased type 2 responses in vivo. This hypothesis is supported by our observation that the levels of IL-4, IL-5, and IL-10 were markedly elevated. Furthermore, CII-specific IgG1 (whose production is enhanced by IL-4 and is therefore indicative of Th2 response) is also significantly increased in transgenic mice 2 wk after immunization, and the high level of IgG1 persists to the late stage of the disease (6 wk postimmunization). Finally, there is a massive eosinophil infiltration in the arthritic joint of transgenic mice, suggesting the contribution of type 2 response to the disease in transgenic mice. Considering that type 2 cells and cytokines were found in CIA before the onset of clinical arthritis (5, 6), we favor the hypothesis that both type 1 and type 2 cells play roles in the pathogenesis of the disease. In this view, accentuation of either subset can aggravate disease as long as the other subset is able to develop during early phases of the Ag response.

An important question from these findings is how an increased type 2 response exacerbated the disease. Based on several lines of evidence from other autoimmune disease models, it is likely that the IL-10 produced by infiltrating T cells in the joint plays a role in the disease pathogenesis. Results with IL-10 transgenic mice provide evidence supporting this hypothesis. Expression of an IL-10 transgene specifically in the pancreatic β cells of NOD mice promoted the development of diabetes (27, 28). Moreover, Th2 cell-induced diabetes in immune-compromised NOD mice is mediated by local IL-10 production, indicating that IL-10 is able to induce local pathology directly (29). It is likely that a second contributory mechanism is the elevated level of IL-4 and IL-5. High levels of IL-4 secreted by type 2 T cells in the joint stimulate the expression of adhesion molecules such as VCAM-1 on endothelial cells as well as the production of chemokines such as (MCP-1) from endothelial cells, resulting in the recruitment of eosinophils and monocytes to the joints. Type 2 cells also secrete IL-5, which stimulates the growth and differentiation of eosinophils and activates mature eosinophils. Consistent with this mechanism, an extensive eosinophil infiltration was observed in the arthritic joint of chimeric receptor transgenic mice. Finally, high levels of IL-4 may promote CII-specific Ab production, thus leading to more severe disease. For instance, expression of an IL-4 transgene under the control of a MHC class I promoter led to increased levels of total IgG, IgG1, and IgE, and spontaneous autoimmune-type disease (22). The expression of our chimeric receptor was restricted to T cells, and T cell and B cell number and ratio appeared normal in both spleen and lymph node (data not shown). However, CII-specific IgG and IgG1 levels are significantly increased, which may partially account for the more severe disease in transgenic mice.

A prior study demonstrated that local IL-4 gene therapy could protect cartilage and bone from erosion despite severe inflammation (21). However, despite enhanced levels of IL-4 and IL-5 and a marked increase in eosinophils in the joint, there is no significant change in cartilage and bone erosion between chimeric receptor transgenic mice and control littermates when paws with equivalent clinical severity were compared. This observation is consistent with our data that the Th1 response is not significantly suppressed (Fig. 4A) in the early stage of the immune response in transgenic mice. Our finding suggests that despite the enhanced Th2 response and the presence of eosinophils, an initially normal Th1 response in chimeric receptor transgenic mice can trigger a cytokine cascade

to activate macrophages/monocytes, which, in turn, stimulates synovial cell proliferation, pannus formation, and destruction of cartilage and bone.

Contributions of the type 2 response to autoimmune disease do not appear to be limited to CIA. Recent work in both diabetes and EAE models has found that adoptive transfer of Ag-specific Th2 cells into immunodeficient mice not only failed to protect against Th1 cell-induced disease, but also caused Th2-related disease. Specifically, infiltration of eosinophils in the islets and infiltration of mast cells in the spinal cord were observed for insulin-dependent diabetes mellitus and EAE, respectively (29, 30). Moreover, adoptive transfer of long term T cell clones with a fixed Th2 cytokine profile caused diabetes in immunocompetent mice (31). Our results demonstrate that even in the absence of adoptive transfer and without an expanded population of Ag-specific T cells, sustained elevation of type 2 responses may not be benign and has the potential to cause clinical disease in animal models.

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