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Prevention of Collagen-Induced Arthritis in Mice Transgenic for the Complement Inhibitor Complement Receptor 1-Related Gene/Protein y

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The objective of these studies was to examine collagen-induced arthritis (CIA) in C57BL/6 mice transgenic for the rodent complement regulatory protein complement receptor 1-related gene/protein y (Crry) (Crry-Tg), a C3 convertase inhibitor. The scores for clinical disease activity and for histological damage in the joints were both significantly decreased in Crry-Tg mice in comparison to wild-type (WT) littermates. The production of both IgG1 and IgG2a anti-collagen Abs was reduced in the Crry-Tg mice, although spleen cell proliferation in response to collagen type II was not altered. The production of IFN-γ, TNF-α, and IL-1β by LPS-stimulated spleen cells was decreased, and IL-10 was increased, in cells from Crry-Tg mice in comparison to WT. The steady-state mRNA levels for IFN-γ, TNF-α, and IL-1β were all decreased in the joints of Crry-Tg mice in comparison to WT. The synovium from Crry-Tg mice without CIA contained the mRNA for the Crry transgene, by RT-PCR, and the synovium from transgenic mice with CIA exhibited little deposition of C3 protein by immunohistological analysis. These results suggest that suppression of CIA in Crry-Tg mice may be due to enhanced synthesis of Crry locally in the joint with decreased production of proinflammatory cytokines. The Journal of Immunology, 2003, 171: 2109–2115.

Collagen-induced arthritis (CIA) in mice and rats is a chronic inflammatory arthritis that resembles the human disease rheumatoid arthritis (1). CIA is induced by injection of bovine type II collagen (CII) in CFA; the mechanism of disease involves both anti-collagen Abs and sensitized lymphocytes in mediating joint destruction. A mild variant of CIA can be induced in naive recipients through the passive transfer of either spleen and lymph node (LN) cells or serum from diseased animals (2, 3). A variety of therapeutic approaches to inhibit humoral, cellular, or inflammatory mechanisms and pathways have been evaluated in CIA.

The complement system is important in mediating events of inflammation and tissue damage in CIA (summarized in Ref. 2). The relative roles of C3 or C5 in CIA have been explored using therapeutic interventions that interfere with one or both of these complement components. In early studies, activation and depletion of C3 by injection of purified cobra venom factor led to a delay in the onset of CIA in rats until C3 levels returned to normal (4). Both IgG and C3 were deposited on the cartilage in rats and mice with CIA (5). Decomplementation with cobra venom factor led to a failure to passively transfer disease with anti-collagen Ig; these rats demonstrated IgG but not C3 on the articular cartilage (6). Inhibition of complement with soluble complement receptor 1 (sCR1), a potent inactivator of both C3 and C5 convertases, delayed the onset and progression of CIA in rats (7). Delivery of sCR1 by gene therapy also inhibited the development and progression of CIA in mice; both anti-CII Ab levels and T cell responses to collagen in vitro were reduced by this treatment (8). Mice deficient in either C3 or factor B were inhibited in the development of CIA after one injection of CII and demonstrated decreased levels of anti-collagen Abs (9). The Ab levels were restored by a second injection of CII and these mice now exhibited CIA, although the arthritis score was low in the C3-deficient mice. C3 deficiency may have affected Ag presentation and induction of an Ab response to CII, in addition to influencing pathways of inflammation and joint damage. However, these results suggest that both the classical and alternative pathways of complement activation may be involved in CIA.

Additional studies have explored the role of C5 in CIA. C5-deficient SWR mice demonstrated high levels of IgG2a Abs to CII but did not develop CIA (10). The results of one study suggested that CIA was absent in SWR mice secondary to a mutation in a Vβ TCR and not to a deficiency in C5 (11). However, two subsequent studies refuted this conclusion, further indicating that the presence of C5 was an absolute requirement for CIA (12, 13). In addition, C5-deficient B10.D2 mice failed to develop CIA despite deposition of both IgG and C3 on the surface of the articular cartilage (14). In more recent studies, treatment of DBA mice with a mAb to murine C5 both prevented the onset of and ameliorated established CIA (15). DBA mice congenic for C5 deficiency also exhibited resistance to induction of CIA despite demonstrating normal humoral and cellular immune responses to collagen (16).

The complement system exhibits intrinsic membrane-bound regulatory proteins that inhibit activation of both the classical and alternative pathways (17). Blockade of both complement receptor 1-related gene/protein y (Crry), a membrane-bound rodent molecule similar in function to human sCR1, and of CD59, a regulator...
of the membrane attack complex, with specific Abs increased synovial proliferation in CIA in rats (18). These results suggested that endogenous regulators of complement may inhibit inflammatory arthritis. Recent studies indicated that treatment of mice with recombinant Cryg Ig lagged to inhibit CIA in comparison to treatment with anti-C5 Abs (19). To further explore the effects of Cryg on CIA, we performed studies in mice transgenic for Cryg (Cryg-Tg), expressing high circulating levels of this complement inhibitor.

Materials and Methods
Preparation of Cryg-Tg mice
Cryg-Tg mice were originally produced on an outbred CD-1 background, as previously described (20). The cDNA encoding the soluble form of mouse Cryg (signal peptide and five short consensus repeats representing the extracellular portion of Cryg) was cloned into the metallothionein (MT) expression vector p2990B4 containing the MT-I promoter, the human growth hormone polyadenylation sequence, and the mouse MT gene 5′ and 3′ flanking regions. Mice expressing the transgene were detected by PCR on tail cultures and by measurement of serum levels with a specific ELISA (Cryg is not normally found in mouse serum) (20). For these studies, mice derived from a single founder were backcrossed into C57BL/6 mice until the tenth generation. The range of serum levels of Crry in the mice used in this work derived from a single founder were backcrossed into C57BL/6 mice until the tenth generation. The range of serum levels of Crry in the mice used in this experiment was 3.92–32.5 μg/ml (mean ± SEM 15.1 ± 3.4, n = 9), comparable to levels previously described in Cryg-Tg mice (20). Littermates bred contemporaneously which lacked the Cryg transgene were used as controls.

Collagen-induced arthritis
Cryg-Tg mice and wild-type (WT) littermates were maintained in the Center for Animal Care, University of Colorado Health Sciences Center (Denver, CO), and were fed standard breeders’ chow. For induction of CIA, 10-wk-old mixed male and female mice were injected intradermally with 100 μl containing 200 μg of bovine CII (Elastin Products, Owensville, MO) and 200 μg of inactivated Mycobacterium tuberculosis (H37Ra; Difco, Detroit, MI) in FDA on days 0 and 21. Between days 21 and 82 the mice were examined three times weekly by two trained laboratory personnel who were blinded to the transgene status and independently assessed the presence of serum levels with a specific ELISA (Cryg is not normally found in mouse serum) (20). For these studies, mice derived from a single founder were backcrossed into C57BL/6 mice until the tenth generation. The range of serum levels of Crry in the mice used in this experiment was 3.92–32.5 μg/ml (mean ± SEM 15.1 ± 3.4, n = 9), comparable to levels previously described in Cryg-Tg mice (20). Littermates bred contemporaneously which lacked the Cryg transgene were used as controls.

Histological examination
On day 82, both forepaws and the right hind limb (including the paw, ankle, and knee) were surgically removed and immediately fixed in 10% buffered formalin (Biochemical Sciences, Swedesboro, NJ). The tissue samples were prepared and histological examination was performed as previously described (21). The joint sections were scored for changes in synovial inflammation, pannus, cartilage damage, and bone damage, all on a scale of 0–5, with an overall score calculated as the total of the four individual parameters. The compiled data for nine transgenic mice and nine WT mice were expressed as the mean ± SEM based upon a set of five joints per animal. Immunohistological analysis for the presence of mouse C3 in synovial tissues from mice with CIA was performed as described (22).

Anti-collagen Abs
Serum was collected from all mice by retro-orbital aspiration of blood on days 0 and 21, before each injection of CII, and by cardiac puncture at the end of the study on day 82. IgG2a and IgG1 Abs to CII were measured in triplicate by specific ELISA as previously described (19, 23). A standard pool of anti-collagen Abs was obtained by combining sera from several mice with severe CIA; the levels of IgG1 and IgG2a Abs in this pool were considered to be 100 U/ml. A standard curve was obtained using serum dilutions and the Michaelis-Menton equation was used to convert OD values into units. The anti-collagen Ab data were expressed as the difference in levels between days 21 and 82.

T cell proliferation
In vitro proliferation of spleen and regional LN cells was examined as recently described (19, 23). LN cells were removed from mice at day 82 and single cell suspensions of 5 × 10⁶ cells/well were cultured in a flat-bottom 96-well plate at 37°C in supplemented RPMI 1640 medium with 5% CO₂. The cells were cultured for 72 h with no further stimulus (medium alone), with 50 μg/ml heat-denatured CII, or with 1 μg/ml PHA. The cells were pulsed with [³H]thymidine for an additional 24 h and then harvested with a semiautomatic measurement of [³H]thymidine incorporation. The mean cpm was calculated, and the results were expressed as the stimulation index (SI, mean ± SEM) as follows: SI = cpm from cells cultured with CII or PHA divided by cpm from cells cultured with medium alone.

Cytokine assays
Cytokine production was measured in spleen cells obtained on day 82. Single cell suspensions (2 × 10⁶ cells/well) were incubated in triplicate for 48 h at 37°C with 50 μg/ml CII, 5 μg/ml LPS, or medium alone. Cytokines in spleen cell supernatants were measured using specific ELISAs as previously described (19, 23). The lower limit of sensitivities for these ELISAs were: IFN-γ and IL-10, 30 pg/ml; IL-1β and TNF-α, 15 pg/ml; and IL-1 receptor antagonist (IL-1Ra), 150 pg/ml.

Cytokine mRNA levels in joints
An RNase protection assay was used to measure levels of various cytokine mRNA in knees dissected from mice at day 82, as recently described (19, 23). Total RNA was extracted from the joints using TRizol (Life Technologies, Gaithersburg, MD) and the quality of the RNA was determined by agarose gel electrophoresis. Synthesis of [³²P]UTP-labeled antisense RNA probes was conducted using two mouse cytokine multiprobe template sets (nick-2b and nick-3b; RibonQuant, BD Pha/Mingen, San Diego, CA) and the MaxiScript in vitro transcription kit (Ambion, Austin, TX). Twenty micrograms of intact RNA from each joint were hybridized with the [³²P]-labeled probes using the RPAIII kit (Ambion) followed by separation of the protected probes on 6% polyacrylamide/7 M Tris borate EDTA/urea gels (1 mm; Invitrogen, Carlsbad, CA). The Tris borate EDTA/urea gel was transferred to chromatography filter paper and vacuum-dried for 30 min at 70°C before exposing to a PhosphorImager screen for 48 h. A Storm PhosphorImager (Molecular Dynamics, Sunnyvale, CA) was used to visualize the gel, followed by analysis of digitized data on density of the individual bands. The densities of cytokine mRNA bands were expressed as ratios of mRNA for G3PDH determined in each gel.

RT-PCR for Cryg transgene mRNA in mouse tissues
RNA was isolated from different tissues of Cryg-Tg and WT mice without CIA using TRizol reagent followed by phenol-chloroform extraction. Total RNA was denatured by incubation at 65°C for 10 min. cDNA was produced from 5 μg of total RNA by reverse transcription using random decamers (50 μM) (Ambion). PCR was performed in tubes containing the generated cDNA, 100 μM DNTPs, 0.1 μM of each primer, 50 mM MgCl₂, 10 mM Tris buffer, 2.5 U of Taq polymerase, and nuclease-free water. Thirty-one cycles of a 1-min denaturation at 94°C, 1 min annealing at 60°C, and 1 min extension at 72°C were performed. The 5′ primer used in PCR for the Cryg transgene was 5′-CCCT CAC TTA CTC CGT AGC TAC-3′, which hybridizes to bases +33 to +53 of the MT-I promoter, and the 3′ primer was 5′-CAG CAC TTC CAC CGG TTT CAC-3′, which hybridizes to a sequence encoding a portion of the first short consensus repeat of Cryg (20). Thus, these primers will amplify the Cryg transgene and not endogenous Cry in mouse tissues. Control PCR was performed using primers for G3PDH: 5′ primer, 5′-TGA AGG TCG GTG TCA AGC GAT GC-3′; and 3′ primer, 5′-CAT GTA GCC GAT CAG GAT GCC-3′. The sizes of the amplified cDNAs are 464 bp for Cryg and 983 bp for G3PDH. Samples were not run in the absence of the reverse transcription enzyme because samples from WT mice served as negative controls.

Spleen cell populations
The distribution of cells in the spleens obtained at day 82 from transgenic and WT mice were determined by flow cytometric analysis as recently described (23). After washing isolated spleen cells three times with PBS containing 1% heat-inactivated FCS, 1 × 10⁶ cells were incubated for 30 min at 4°C with the following specific Abs: macrophages, rat anti-mouse F4/80 (IgG2b, PE-conjugated; Caltag Laboratories, Burlingame, CA); NK cells, rat anti-mouse pan-NK (IgM, PE-conjugated; Caltag Laboratories); B cells, rat anti-mouse CD19 (IgG2a, FITC-conjugated; Caltag Laboratories); and CD4 (+)/CD3 (+) T cells, rat anti-mouse GK1.5 (IgG2a, FITC-conjugated; BD Biosciences, Mountain View, CA) and rat anti-mouse CD3 (IgG2a, PE-conjugated; Caltag Laboratories). The cells were again washed...
three times with PBS and 2% FCS, fixed in 2% formaldehyde in PBS with 2% FCS, and analyzed on an EPICS XL flow cytometer (BD Biosciences).

**Statistical analyses**

The clinical disease activity scores, T cell proliferation, anti-collagen Ab levels, cytokine levels in spleen cell supernatants, spleen cell populations, and cytokine mRNA levels in joints were all examined using ANOVA with tests for multiple comparisons. W-statistics was used to confirm normal distributions of data, and the Brown and Forsyth test was used to examine differences in variance. Selected comparisons between potentially related data sets were calculated using Pearson’s correlation coefficient. The Student t test was used to analyze the histological data. In all cases, data were expressed as the mean ± SEM, based on nine animals in each group, and p < 0.05 was considered significant.

**Results**

**Effect of Crry on clinical and histological disease activity**

Although DBA mice are most susceptible to CIA, the disease can be induced in C57BL/6 mice with an optimal immunization schedule and a longer period of observation (24). We used CII in IFA with added *M. tuberculosis* for both the initial immunization and the booster injection on day 21. In addition, we followed the mice for 61 days after the second injection for development of clinical evidence of arthritis. The incidence of arthritis was 100% in both transgenic and WT mice. However, the Crry-Tg mice developed less severe arthritis as indicated by lower disease activity scores from day 58 through day 82 in comparison to WT mice (p < 0.05) (Fig. 1). At day 82, the mean disease activity score in the Crry-Tg mice was <40% of the value for the WT mice.

The histological changes of inflammation and joint tissue destruction at day 82 were also reduced at least 70% in the Crry-Tg mice in comparison to the WT (Table I). The scores for inflammation, pannus, cartilage damage, and bone damage, as well as the overall histopathology score, were all significantly lower in the Crry-Tg mice in comparison to the WT (p < 0.05 for each parameter).

**Cellular and humoral immunity to collagen**

To assess the possible effects of the Crry transgene expression on cellular and humoral immune responses to CII, both proliferation of spleen and LN cells and serum levels of IgG1 and IgG2a anti-collagen Abs were measured. Spleens and regional LNs were harvested at day 82 and isolated cells were cultured for 72 h in the presence of 50 μg/ml CII or 1 μg/ml PHA with subsequent measurement of T cell proliferation. The SIs in spleen or LN cells were not different between Crry-Tg and WT mice cultured either with CII or PHA (CII stimulation: WT vs Tg spleen cells, p = 0.48, and WT vs Tg LN cells, p = 0.68; PHA stimulation: WT vs Tg spleen cells, p = 0.29, and WT vs Tg LN cells, p = 0.37) (Fig. 2). The absolute values of the SIs were rather low because of the long time period between the boost injection of CII and termination of the experiment.

Anti-collagen Abs were not detected in any mouse serum at day 0. However, both IgG1 and IgG2a Abs to CII were present at days 21 and 82. The absolute serum levels of IgG1 and IgG2a anti-collagen Abs were the same in both Crry-Tg and WT mice at day 21 (IgG1: WT 23.8 ± 9.9 U/ml, Tg 30.0 ± 12.6 U/ml; IgG2a: WT 27.7 ± 8.4 U/ml, Tg 30.8 ± 7.8 U/ml). The absolute serum levels of both IgG1 and IgG2a anti-collagen Abs at day 82 were increased over day 21 in both groups of mice but the changes between days 21 and 82 were less in the Tg mice (IgG1: WT 187.5 ± 44.1 U/ml, Tg 84.8 ± 28.8 U/ml, p < 0.05; IgG2a: WT 99.7 ± 23.8 U/ml, Tg 49.7 ± 8.3 U/ml, p < 0.05). The changes in both IgG1 and IgG2a anti-collagen Ab levels between days 21 and 82 are expressed in Fig. 3 and were lower in the Crry-Tg mice in comparison to the WT (p < 0.05 for both comparisons).

![Image](Image_1.png)

**FIGURE 1.** Clinical disease activity of CIA in Crry-Tg and WT mice. Mice were immunized with CII in IFA with added *M. tuberculosis* at days 0 and 21. The clinical disease activity was assessed three times weekly between days 21 and 82 using a 3-point score for each paw and four paws per animal (maximum score of 12). The data are expressed as the clinical disease activity score of Crry-Tg (□) and WT (○) mice vs days after the initial collagen injection. The data represent mean ± SEM with n = 9 in each group.

![Image](Image_2.png)

**FIGURE 2.** Proliferation of spleen or LN cells obtained from Crry-Tg and WT mice. Spleens and regional LN were obtained from mice with CIA at day 82 and single cell suspensions were incubated with 50 μg/ml CII (□) or 1 μg/ml PHA (■) for 72 h. [3H]Thymidine incorporation was assayed over a subsequent 24 h and cpm was measured. The data are expressed as SI: cpm in the presence of stimulant/cpm in medium alone (mean ± SEM with n = 9 in each group).
Cytokine production by spleen cells

Spleen cells were cultured with medium alone, 50 μg/ml CII, or 5 μg/ml LPS for 48 h and cytokine levels in the supernatants were measured by ELISA. The levels of IFN-γ, IL-1β, and TNF-α were all decreased in the supernatants of LPS-stimulated cells from Crry-Tg mice in comparison with WT (Fig. 4, p < 0.003 for each comparison). IL-1β production also was reduced in cells from CII-stimulated spleen cells from Crry-Tg mice (p < 0.03). In contrast, the levels of IL-10 were increased in the supernatants of LPS-stimulated cells from Crry-Tg mice compared with WT (p < 0.03). The levels of IL-1Ra were high in supernatants from all cells cultured with LPS and were not different between the two groups of mice. An analysis of spleen cell subpopulations by cell cytometry indicated no difference in the absolute number of macrophages between Crry-Tg and WT mice (data not shown).

Cytokine mRNA levels in joints

To determine the effects of high levels of soluble Crry on cytokine production in joints from mice with CIA, steady-state levels of various cytokine mRNA were determined in isolated whole joints. The data were expressed as the ratio of cytokine mRNA to G3PDH mRNA (Table II). Significant decreases in steady-state levels of mRNA were observed in the joints of Crry-Tg mice with CIA in comparison to WT mice for the following cytokines: IFN-γ, TNF-α, IL-1β, TNF-β, and TGF-β1. The steady-state mRNA level for IL-1Ra was increased in Crry-Tg mice with CIA in comparison to WT.

Crry mRNA in tissues

The presence of mRNA for the Crry transgene was determined by RT-PCR in RNA extracted from whole knee joints of Crry-Tg and WT mice with CIA, and from muscle, skin, kidney, spleen, and synovium from Crry-Tg and WT mice without CIA. The whole knee joint included synovium, bone, articular cartilage, bone marrow, joint capsule, skin, and muscle. The knees of Crry-Tg mice with CIA showed abundant presence of the transgene mRNA while none was detected in the joints of WT mice (Fig. 5A). All of the examined organs from Crry-Tg mice without CIA demonstrated the presence of the transgene mRNA whereas it was not detected in the tissues from WT mice (Fig. 5B). The levels of control mRNA for G3PDH were the same in all tissues from both Crry-Tg and WT mice. Because the whole joint contained multiple tissues, it was of interest to examine isolated synovia from mice without CIA, as the synovium is the primary tissue involved in the mechanisms of damage in inflammatory arthritis. The synovium of mice without CIA clearly showed presence of the Crry transgene in the Tg mice whereas it was absent from the WT mice (Fig. 5C).

Immunohistochemical studies

The synovial tissues obtained from mice with CIA at day 82 were studied by immunohistochemistry using an Ab specific for mouse C3. The joints from the WT mice with CIA were largely destroyed while joints from the Crry-Tg mice demonstrated little inflammation and destruction (Fig. 5, A and B). Staining for C3 was present throughout the inflamed synovium of WT mice but was barely visible in joints from the Crry-Tg mice with CIA (Fig. 6, C and D). In the WT mice, C3 was located both superficially and deep in the...
synovium. These results suggested that the presence of the transgenic Crry protein locally in the joints during the development of CIA greatly reduced the deposition of C3.

Discussion
The results of these studies indicated that mice transgenic for the rodent complement inhibitor Crry were largely protected from developing CIA. In comparison to WT mice, the Crry-Tg mice demonstrated significant decreases in both clinical disease activity and histological changes in the joints. The Crry-Tg mice with CIA displayed suppressed production of both IgG1 and IgG2a anti-collagen Abs between days 21 and 82, but demonstrated no differences with the WT mice in LN or spleen cell proliferation after collagen stimulation. Production of the inflammatory cytokines IFN-γ, TNF-α, and IL-1β was decreased in LPS-stimulated cultured spleen cells from the Crry-Tg mice. This cytokine production was presumably by macrophages and the percentages of these cells in the spleen were not different between Crry-Tg and WT mice. Steady-state mRNA levels for these same cytokines were decreased in the joints, all in comparison with WT mice. Lastly, mRNA for the Crry transgene was produced locally in the synovium of mice without CIA and little C3 was deposited in the joints of Crry-Tg mice with CIA.

The mice transgenic for Crry exhibited more inhibition of CIA than was recently observed in mice treated with a recombinant Crry-Ig fusion protein (19). In the previous studies, mice received 3-mg injections of Crry-Ig i.p. every other day for 2 wk. Those mice demonstrated no decreases in clinical disease activity or in histological changes in the joints, although collagen-induced proliferation of spleen cells and IgG2a anti-collagen Ab production were both decreased by treatment with the Crry-Ig protein. Moreover, steady-state mRNA levels for TNF-α and IL-1β were significantly decreased in the joints of mice treated with anti-C5 Abs, which demonstrated significant inhibition of clinical and histological disease, in comparison to mice receiving Crry-Ig (19).

Crry may have been more effective as an exogenously produced transgenic protein than delivered as a recombinant protein primarily because of enhanced local production in the joints. The serum levels may not have been markedly different between the two studies. Although we did not perform pharmacokinetic experiments, an injection of 3 mg of Crry-Ig i.p. every 48 h should have led to a trough in serum levels of 15–20 μg/ml (19, 25). In the present studies with Crry-Tg mice, the mean serum level was 15 μg/ml. An alternative explanation for the beneficial effects of Crry in the transgenic mice would be that constant levels of the protein were present throughout the experiment. Perhaps the presence of soluble Crry from the time of the first immunization with collagen in the Crry-Tg mice altered B cell Ag presentation, thus preventing initiation of the arthritis. Lastly, soluble Crry in the transgenic mice may have been more biologically active than the Fc fusion protein, although the latter was quite effective in blocking Ab-induced glomerulonephritis (25).

There are several findings in these and our previous studies that are relevant to the role of complement in inflammatory arthritis. In the experiments reported herein with Crry-Tg mice, as well as in DBA mice treated with anti-C5 mAb, the levels of proinflammatory cytokines in the joint were substantially decreased in treated
or transgenic mice in comparison to controls. This result suggests that complement activation may itself lead to proinflammatory cytokine production. This production may occur either through activities of the membrane attack complex, interactions of C5a with its receptor, or chemotaxis and attraction of activated neutrophils to the joint that then produce TNF-α, IL-1β, and other cytokines. Of interest, similar findings have been reported in a model of experimental myocarditis where the lack of C3 resulted in decreased cytokine production in splenocytes and in a substantial decrease in myocardial inflammation (26).

Increased IL-10 production by splenic macrophages may also have contributed to the inhibition of CIA. IL-10 blocked monocyte production of the proinflammatory cytokines IL-1, IL-6, IL-8, TNF-α, GM-CSF, and G-CSF at the transcriptional level (27). IL-10 also inhibited metalloproteinase and stimulated inhibitor production in macrophages (28). However, the mechanism of priming of splenic macrophages to produce enhanced levels of IL-10 after LPS stimulation in vitro in Crry-Tg mice in our studies remains unknown. Increased IL-10 production by Ag-stimulated spleen cells was observed in CR1- and CR2-deficient mice in studies on experimental autoimmune myocarditis (26). Perhaps complement deficiency leads to enhanced IL-10 production by macrophages or T cells through cytokine-mediated immunoregulatory circuits (29, 30).

The decrease in anti-collagen Abs found in Crry-Tg mice may be secondary to the inhibitory effects of Crry on C3 activation, leading to less interaction between complement receptors CD21 and CD35 on B cells and follicular dendritic cells, and to less B cell coactivation through the CD19/CD81 complex (31). This possible effect of Crry is consistent with the marked decrease in T-dependent IgG responses in mice lacking either C3 (32) or CD21/CD35 (33), and is also similar to findings in the experimental myocarditis model (26). Thus, the effects of Crry in CIA may be due both to inhibition of B cell function as well as to blockade of production of proinflammatory cytokines.

The results of our studies do not bear on the issue of relative importance of inhibiting C3 vs C5 in the treatment of disease. Although Crry is a specific murine C3 convertase inhibitor, inhibition of C5a generation will result from decreased activation of the remainder of the cytokine pathway in the presence of Crry. The results of recent studies indicate a primary importance for C5a in inflammatory disease. Mice deficient in the C5a receptor demonstrated a complete protection from arthritis induced by injection of monoclonal anti-collagen Abs whereas arthritis was not reduced in C3a receptor-deficient mice (34). In addition to functioning as a potent chemoattractant, C5a may further participate in inflammatory diseases through induction of activating FcγRIII and suppression of inhibitory FcγRII (35).

More effective suppression of the complement system in disease may result from enhanced levels of complement regulatory proteins locally in tissues. Endogenous expression of complement regulatory proteins appears to be important in resistance to inflammatory disease as blockade of both Crry and CD59 led to more severe CIA in rats (18). The results of earlier studies indicated that endogenous expression of CD59 may play a role in protection against synovial injury mediated by the membrane attack complex as inhibition of CD59 led to an acute, transient arthritis (36). The importance of local complement regulatory proteins is further supported by the beneficial effect of an intra-articular injection of a truncated form of human CR1 in Ag-induced arthritis in rats (37). Intra-articular injection of an Ig fusion protein of the complement regulatory protein DAF also led to a reduction in the severity of Ag-induced arthritis in rats (38).

In summary, the results of our studies indicate that mice transgenic for the murine C3 convertase inhibitor Crry demonstrated decreased clinical activity and histological damage in CIA, a mouse model of inflammatory arthritis resembling rheumatoid arthritis. The enhanced efficacy of Crry expressed as a transgenic protein rather than administered as a recombinant protein may be
due primarily to increased local production in the joint. This conclusion further supports efforts to develop new therapeutic approaches to rheumatoid arthritis based on specific delivery to, or expression of, novel agents in the joint.

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