Collagen-induced Arthritis in the BB Rat

Prevention of Disease by Treatment with CTLA-4-Ig

Debbie Barney Knoerzer, Robert W. Karr, Benjamin D. Schwartz, and Laurel J. Mengle-Gaw
Department of Immunology, Discovery Research, G.D. Searle and Co., St. Louis, Missouri 63198

Abstract

Antigen-specific T cell activation requires two independent signalling events, one mediated through T cell receptor engagement by the antigen-presenting cell–expressed peptide/class II major histocompatibility complex, and the second through the cognate interactions of costimulatory molecules expressed on the T cell and antigen-presenting cell. There is evidence from in vitro and in vivo experimental systems suggesting that the CD28/B7 costimulatory pathway is crucial for induction of maximal T cell proliferation and T helper-B cell collaboration for IgG production. This pathway can be blocked by CTLA-4-Ig, a soluble form of CTLA-4 which binds with high avidity to the CD28 ligands, B7-1 and B7-2. Here, we show that CTLA-4-Ig treatment prevents clinical and histological manifestations of disease in a collagen-induced arthritis model of rheumatoid arthritis in the diabetes resistant BB/Wor rat, when therapy is initiated before immunization with bovine type II collagen (BIIC). Anti-BIIC antibody titers are reduced in CTLA-4-Ig–treated rats compared to diseased control animals. Histologically, joints from CTLA-4-Ig–treated animals show no histological abnormalities, in contrast to control antibody-treated animals, which show complete erosion of the articular cartilage and bone. Despite the efficacy of CTLA-4-Ig in preventing clinical and histological signs of arthritis and reducing antibody responses to BIIC, delayed type hypersensitivity responses to collagen 18 d or more after CTLA-4-Ig treatment ends are similar in CTLA-4-Ig–treated and untreated rats, suggesting that the prolonged disease suppression observed does not result from induction of T cell anergy. (*J. Clin. Invest.* 1995. 96:987–993.) Key words: immunosuppression • rheumatoid arthritis • costimulation • anergy

Introduction

Optimal activation of antigen-specific T cells requires the induction of a nonspecific costimulatory signalling pathway, in addition to the primary antigen-specific signal delivered by T cell receptor engagement (1, 2). One such costimulatory accessory molecule is the T cell surface antigen, CD28, whose interaction with its antigen-presenting cell–expressed ligands B7-1 and B7-2 during TCR engagement has been shown to be crucial for maximal T cell signalling (3). B7 ligand binding to CD28 costimulates T cell proliferation and IL-2 transcription (4–6), and monoclonal antibodies directed against both B7 and CD28 specifically block T helper (T_h)-mediated Ig production by B cells in vitro (7). These observations demonstrate the importance of the CD28/B7 costimulus to the functional collaboration between T_h and B cells. The CD28/B7 costimulatory pathway can also be blocked with a chimeric Ig fusion protein of CTLA-4, a CD28 homologue which binds B7-1 and B7-2 with high avidity (8, 9). CTLA-4-Ig binding to B7 has been shown to potently inhibit both T_h proliferation and Ig secretion by B cells in vitro (8).

Antigen stimulation through the TCR in the absence of costimulation can lead to antigen-specific hyporesponsiveness or clonal T cell anergy in vitro (3). For this reason, there has been much interest recently in identifying therapeutic approaches aimed at blocking costimulation, especially in the areas of tissue transplantation and autoimmunity. Because there is no human–rodent species barrier to CTLA-4/B7 interaction, the effects of CD28/B7 pathway blockade using human CTLA-4-Ig constructs have been studied in a variety of rodent-based disease models. In recent in vivo experiments, CTLA-4-Ig treatment has been shown to suppress primary, and to a lesser extent, secondary antibody responses to sheep erythrocytes and keyhole limpet hemocyanin immunogens (10), block xenogeneic pancreatic islet rejection in mice and induce long-term, donor-specific tolerance to the graft (11), increase cardiac allograft survival in rats (12), and block autoantibody production, and prolong survival of mice affected by systemic lupus erythematosis (13). The induction of anergy, rather than prolonged immunosuppression, by CTLA-4-Ig in these systems is variable, and may depend upon the potency of the immunogen, the use of species-matched CTLA-4-Ig, or a variable dependence of certain T cell populations on B7 costimulation.

The diabetes-resistant (DR) BB/Wor rat is a subline of the diabetes-prone BB rat which develops severe, aggressive arthritis upon immunization with heterologous native type II collagen (14). Arthritis develops bilaterally with 100% incidence in the hindpaws, with clinical signs beginning at day +10 after a single collagen injection. Significantly, the DR BB/Wor rat has a major histocompatibility complex (MHC) genetic background relevant to that predisposing to human RA; it shares with RA-susceptible humans a homologous MHC class II–encoded arthritis susceptibility epitope (14). Progression of the disease is associated with infiltration into the periarticular space of mononuclear and multinucleated inflammatory cells and re-

Address correspondence to Laurel J. Mengle-Gaw, Department of Immunology, 700 Chesterfield Village Parkway, Chesterfield, MO 63198. Phone: 314-537-7031; FAX: 314-537-6776.

Received for publication 16 December 1994 and accepted in revised form 5 May 1995.

J. Clin. Invest.

0021-9738/95/08/0987/07 $2.00
Volume 96, August 1995, 987–993

1. *Abbreviations used in this paper:* BIIC, bovine type II collagen; DR, diabetes resistant; DTH, delayed type hypersensitivity; IFA, incomplete Freund's adjuvant.

sorption of articular cartilage and bone (14). Histologically, DR BB/Wor collagen-induced arthritis resembles human rheumatoid arthritis: multinucleated giant cells, pallisading cells, and pregranulomatous nodules are seen in sections of severely affected joints (Barney Knoerzer, D., B. D. Schwartz, and L. J. Mengle-Gaw, unpublished observations). Antibody mechanisms have been implicated in the pathogenesis of arthritis in this model (14).

In this report, we show that administration of soluble CTLA-4-Ig prevents development of clinical manifestations of collagen-induced arthritis in the DR BB/Wor rat during observation periods up to 62 d. These results demonstrate for the first time that blockade of the CD28/B7 costimulation pathway prevents disease in an in vivo arthritis model.

Methods

Animals. DR BB/Wor rats were obtained from the University of Massachusetts, Worcester, MA, breeding colony. Equal numbers of males and females, aged 45–65 d (~ 150 grams), were used in these studies.

Preparation of CTLA-4-Ig and control Ig. A soluble fusion protein consisting of the extracellular domain of human CTLA-4 and a mouse IgG2a Fc was prepared. The Fc region of mouse IgG2a was generated by reverse transcription followed by PCR amplification using polyA+ RNA isolated from the L243 hybridoma (ATCC #HB 55; American Type Culture Collection, Rockville, MD) and the IgG2a-specific sense primer 5′-GATCGGATCCGAGCCCAGAGGGCCCACAATCAA GCC-3′ and antisense primer 5′-GATCAAGCTTAGATCTTATCA-TTTACCCGGAGTCCGGGAGAA-3′ (15). The PCR product was digested with BamHI and BglII and cloned into the BamHI site of pMON3360B (16) yielding plasmid pMON24210. DNA corresponding to the signal peptide and extracellular domain of CTLA-4 was generated by PCR amplification using a thymus sDNA library (HL1074a; Clontech, Palo Alto, CA) as template and the CTLA-4–specific sense primer 5′-CCATGGATCCATGGCTTGCCTTGGATTTCAG-3′ and the antisense primer 5′-GATCGGATCCGAAGTCAGAATCTGGGCACG-GTTC-3′ (17). The purified BamHI fragment was cloned into the BamHI site of pMON24210 yielding transfection vector pMON24218. Transfection and isolation of a stable cell line expressing 2–5 μg/ml of CTLA-4-Ig were performed as described (16), using Western blot analysis developed with goat anti–mouse Fc to identify the recombinant protein. Recombinant CTLA-4-Ig was purified using protein A-affinity chromatography. An ascites-purified mouse myeloma IgG2a antibody, UPC-10 (Sigma Chemical Co., St. Louis, MO), directed against β1-6 fructosan, was used as a control. Rats were injected intraperitoneally with a 1-mg/kg dose of CTLA-4-Ig or UPC-10 control antibody (1 mg/ml in PBS) at days −1, 0, +2, +4, +6, +8, and +10 (total of seven doses), relative to bovine type II collagen (BIIC) immunization at day 0. Six rats per antibody treatment group were followed through these studies. Data from one of three experiments giving identical results are reported here.

DR BB/Wor rat immunization with bovine II collagen. Bovine IIC was supplied by Dr. Marie Griffiths, University of Utah, Salt Lake City, UT (18). Rats were injected intradermally at the base of the tail with 100 μg BIIC emulsified in incomplete Freund's adjuvant (IFA) (Difco Laboratories Inc., Detroit, MI). The emulsion was made by homogenizing one part native BIIC (4 mg/ml in 0.1 M acetic acid) into one part IFA at 4°C.

Clinical scoring of arthritis in BIIC-immunized DR BB/Wor rats. Clinical severity of arthritis development in the rat paws was scored on a subjective scale ranging from 1–4 for each paw: 1-redness; 2-swelling; 3-digit deformity; 4-ankle deformity (full ankylosis). All clinical score measurements were performed without knowledge of the treatment regimen the rats received.

Determination of serum anticollagen antibody titers. Sera for determination of IgG anti-BIIC antibody titers were obtained by retroorbital bleeding on various days after collagen immunization or from naive age-matched animals. Serum samples were stored at −20°C and were heat inactivated before testing by ELISA. Briefly, microtiter plates (Nunc Immuno Maxisorp, Kirkegard and Perry Laboratories, Inc., Gaithersburg, MD) were coated with 0.1 ml of 0.01 mg/ml native BIIC in phosphate buffer at 4°C overnight. After washing, the wells were blocked with ELISA buffer (1% BSA in PBS). Serum samples, diluted 1:500, 1:2,500, and 1:5,000 in ELISA buffer, were added to wells and incubated 2 h at 25°C. BIIC-bound serum IgG were detected by a horseradish peroxidase–conjugated goat anti–rat IgG (Accurate Chemical and Scientific Corp., Westbury, NY), and plates were developed using ABTS peroxidase substrate (Kirkegard and Perry Laboratories, Inc.) and read at 405 nm on an automated microplate reader (model 3550; Bio-Rad Laboratories, Richmond, CA). Results are expressed as OD units (experimental wells minus baseline OD). A serum with known high levels of anti-BIIC antibodies was used to generate a highly reproducible standard curve as part of each experiment.

Histology. Relevant paws taken from animals killed at the end of the study were skinned and placed in 10% buffered phosphate formalin (Fisher Scientific Co., Pittsburgh, PA) for > 1 wk before being subjected to acid decalcification (19). Decalcified paws were embedded in paraffin, longitudinally sectioned through the center of the tibia–tarsal joint (Histo Techniques, Powell, OH), and stained with hematoxylin and eosin (19).

Delayed type hypersensitivity (DTH) analysis. Lyophilized bovine type II collagen was prepared by the method of Griffiths et al. (18). 2 μg in 0.05 ml was injected intradermally at shaved sites on the back at various days after collagen immunization. Saline buffer injections (0.05 ml) were used as controls. The injection sites were scored and measured at 48 h. Reactive sites were measured in two directions with calipers, and results expressed as mean±SD diameter of induration.

Statistical analysis. A two-factor repeated measures analysis of variance was performed using the PROC MIXED program in SAS (SAS Institute, Inc., Cary, NC) to compare the anti-BIIC antibody levels and DTH responses in the two treatment groups (CTLA-4-Ig or UPC-10) at each day of measurement.

Results

CTLA-4-Ig administration prevents collagen-induced arthritis in the DR BB/Wor rat. The arthritis which develops in the DR BB/Wor rat after a single intradermal injection of heterologous type II collagen shows a rapid clinical and histological progression (14; Barney Knoerzer, D., B. D. Schwartz, and L. J. Mengle-Gaw, unpublished observations). Early clinical signs of hindpaw disease appear at day +10 after BIIC immunization, with redness in one or both hindpaws, progressing to full hindpaw ankylosis by day +16 to day +18 in 100% of immunized rats. Clinical signs of arthritis can also appear in the forepaws of 20–80% of immunized animals, but forepaw disease is generally less severe than hindpaw disease and its development follows hindpaw disease in affected rats (Barney Knoerzer, D., B. D. Schwartz, and L. J. Mengle-Gaw, unpublished observations).

Fig. 1 *A* shows the result of administration of CTLA-4-Ig to six rats, beginning at day −1, with bovine type II collagen immunization on day 0. CTLA-4-Ig treatment was continued 3–4 times weekly until day +10, when all treatment was discontinued. Animals were followed clinically to day +50 in this experiment. At this dose of CTLA-4-Ig, none of the six rats showed any clinical signs of arthritis during the observation period. This outcome compares dramatically to arthritis development in the UPC-10 antibody–treated control group, where, as with saline-treated control animals (data not shown), aggressive hindpaw arthritis began at day +10 and progressed to full

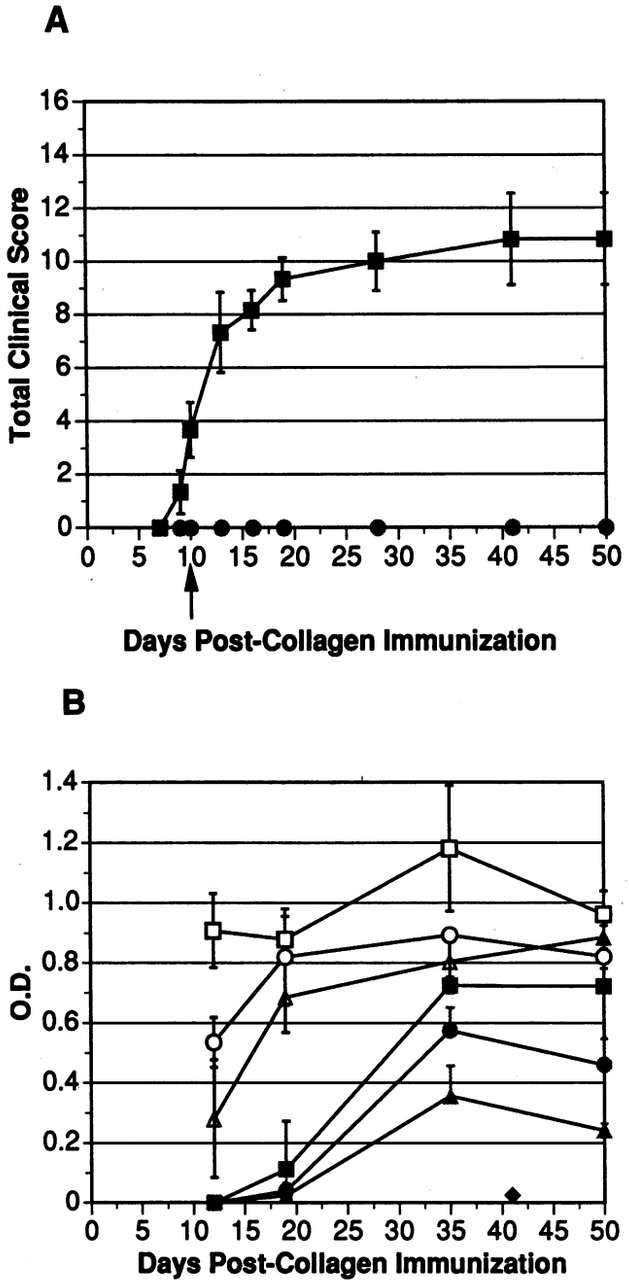

Figure 1. (*A*) Effect of CTLA-4-Ig treatment on the clinical course of arthritis progression in DR BB rats. All rats were immunized with bovine type II collagen at day 0. (— ■ —) Mean clinical score of six control rats treated with UPC-10 antibody; (—●—) Mean clinical score of six rats treated with CTLA-4-Ig. A total of seven doses of either antibody was given between day −1 and day +10. Arrow indicates day of treatment cessation. (*B*) Mean anti-BIIC IgG antibody titers, measured by ELISA at three serum dilutions, of three of the six rats treated with UPC-10 (— □ — 1:500; — ○ — 1:2500; — △ — 1:5000) or CTLA-4-Ig (—■— 1:500; —●— 1:2500; —▲— 1:5000). (— ♦ —) Mean anti-BIIC antibody titer of naive BB littermates.

ankylosis by day +16 (Fig. 1 *A*). Forepaw involvement in the UPC-10–treated animals began at day +19, raising the total clinical score above eight. The data presented are from one of three separate experiments giving identical results. In the other two experiments, four of four and four of four CTLA-4-Ig–treated animals had clinical scores of zero through the 47 and 62-d observation periods, respectively, whereas all of the eight UPC-10–treated rats developed arthritis by day +12 (data not shown).

Humoral response to bovine type II collagen in CTLA-4-Ig–treated rats. Sera from three rats in each of the CTLA-4-Ig and UPC-10–treated groups were analyzed by ELISA for anti-BIIC IgG antibodies after collagen immunization. These results from three serum dilutions at multiple time points are shown in Fig. 1 *B*. Anti-BIIC antibody levels of UPC-10–treated control animals rose rapidly during the first 19 d, in parallel with the development of arthritis in these animals, then persisted at high levels during the remainder of the observation period. Although antibody levels in CTLA-4-Ig–treated rats also rose over time, the antibodies consistently appeared later than in the control-treated rats and the levels were significantly lower at each time point analyzed than in the control-treated rats ($P < 0.05$). Sera from naive age-matched rats have a mean OD of 0.025 at day +41 (Fig. 1 *B*). Anti-BIIC antibody levels were also measured at three serum dilutions in day +12, +19, and +48 samples from three UPC-10–treated rats and three CTLA-4-Ig–treated rats from another experiment and similar results were obtained (data not shown). IgG2a antibodies directed against the CB11 fragment of type II collagen have been identified in the sera of diseased BBN rats (14), and we have previously observed a positive correlation between anti-BIIC titer and disease development in this model (Barney Knoerzer, D., B. D. Schwartz, and L. J. Mengle-Gaw, unpublished observations), suggesting that disease may be mediated at least in part by humoral responses to the heterologous type II collagen used as immunogen. This contention is supported by the observation that mild arthritis can be transferred to naive animals with serum from diseased animals (Anderson, G., D. Barney Knoerzer, B. D. Schwartz, and L. J. Mengle-Gaw, unpublished observations).

Histological analysis of CTLA-4-Ig–treated joints. Hindpaw joints from immunized CTLA-4-Ig–treated rats were examined at day +47 by light microscopy and compared to joints from normal, unimmunized rats, and immunized rats (day +47) receiving UPC-10 control antibody treatment. These sections are compared in Fig. 2. Compared to the normal joint architecture seen in unimmunized animals (Fig. 2 *A*), the joints from BIIC-immunized, UPC-10 control–treated rats show significant pannus formation and complete erosion of cartilage and bone at day +47 (Fig. 2 *B*). By contrast, joints from BIIC-immunized, CTLA-4-Ig–treated rats show no histological abnormalities at day +47 (Fig. 2 *C*).

Cellular response to bovine type II collagen in CTLA-4-Ig–treated rats. Skin delayed type hypersensitivity responses to BIIC were measured in CTLA-4-Ig-treated and control animals (Table I). 4 rats/treatment group were assessed at day +22 and +28 after collagen immunization. The same rats were used for DTH measurement at both day +22 and +28, and were a subset of those whose clinical scores and anti-BIIC antibody titers are shown in Fig. 1. At day +22, the mean DTH score for the CTLA-4-Ig–treated animals was 0.9, with three of the four animals showing no DTH response; this compares with mean DTH scores of 5.2 and 4.7 for the UPC-10–treated and saline-treated control groups, respectively, and represents a significant difference in the ability of CTLA-4-Ig vs UPC-10–treated rats to generate DTH responses to collagen at day +22 ($P < 0.005$). By contrast, the DTH responses at day +28 of

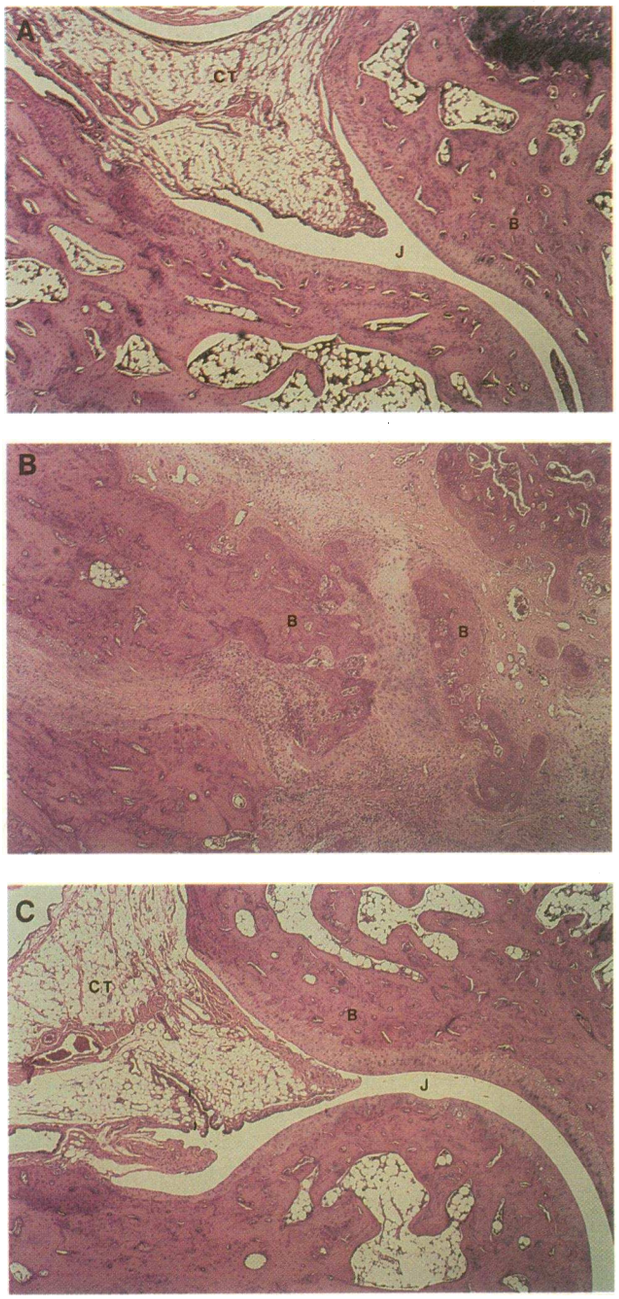

Figure 2. Histological evaluation of the effect of CTLA-4-Ig treatment on arthritis development in the tibia–tarsal joints of BIIC-immunized DR BB rats. Light micrographs were taken of hematoxylin and eosin–stained sections from (*A*) normal, (*B*) UPC-10-treated, arthritic, and (*C*) CTLA-4-Ig–treated DR BB rats 47 d after immunization with bovine CII in IFA. B, bone; J, joint space; CT, connective tissue.

the CTLA-4-Ig–treated animals (mean score = 5.1) are the same as those of control rats receiving either saline (mean score = 4.6) or UPC-10 antibody treatment (mean score = 5.3) (Table I). Therefore, although three of four CTLA-4-Ig–treated animals lack DTH responses to BIIC early after CTLA-4-Ig treatment ends, treated animals regain their ability to generate antigen-specific DTH responses with time; at day +28, CTLA-4-Ig-treated rats show no clinical signs of disease, but do generate normal DTH responses to BIIC. To address the possibility

Table I. Cellular Immunity to Bovine Type II Collagen in DR BB Rats

Treatment Group	Day +22		Day +28	
	No. positive*/ No. tested	Mean diameter‡ of induration±SD	No. positive*/ No. tested	Mean diameter‡ of induration±SD
		mm		*mm*
BIIC + saline	2/2	4.7±0.3	2/2	4.6±0.9
BIIC + CTLA-4-Fc	1/4	0.9±1.9	4/4	5.1±1.3
BIIC + UPC-10	4/4	5.2±0.7	4/4	5.3±1.3
Saline alone	0/2	0.0±0.0	0/2	0.0±0.0

Groups of rats were immunized with BIIC and treated with UPC-10 or CTLA-4-Fc as described in Methods, except the rats in the saline-treated group, who were neither immunized nor treated, and who received saline alone. DTH responses were measured at day +22 and day +28 on the same animals and expressed as the mean±SD diameter of induration (*mm*). * Positive DTH response: mean diameter of induration >3 mm. ‡ Mean±SD calculated using all tested animals in each treatment group.

that the positive DTH responses at day +28 were due to antigen priming with collagen during DTH testing at day +22, additional experiments were done: In two separate experiments, four of four and six of six CTLA-4-Ig–treated rats without arthritis had positive DTH responses to collagen on first testing at day +29 or +36, respectively (data not shown). No CTLA-4-Ig–treated rats developed arthritis following DTH testing.

Collagen rechallenge. In pilot studies, we observed that a CTLA-4-Ig–treated rat without arthritis at day +33 after initial collagen immunization developed robust arthritis after rechallenge with collagen and IFA at day +33. We subsequently conducted a collagen rechallenge experiment with and without the administration of CTLA-4-Ig during the rechallenge, using the six CTLA-4-Ig–treated rats shown in Fig. 1 *A*. At day +50 after the initial collagen immunization and CTLA-4-Ig treatment, each of the CTLA-4-Ig–treated rats had a clinical score of zero. The CTLA-4-Ig–treated rats were divided into two treatment groups in association with collagen rechallenge (BIIC 100 μg in IFA) at day +50: two rats received no additional CTLA-4-Ig treatment, and four rats received seven doses of CTLA-4-Ig during days +47 to +56 (Fig. 3). The untreated

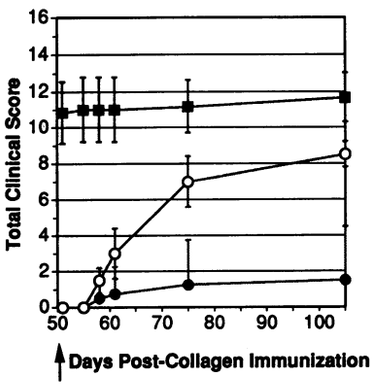

Figure 3. Effect of CTLA-4-Ig treatment of clinical course of arthritis after collagen rechallenge. Rats were rechallenged with collagen at day +50 (*Arrow*). (— ○ —) Rats rechallenged with collagen without additional CTLA-4-Ig treatment (n = 2), (—●—) rats rechallenged with collagen while receiving CTLA-4-Ig treatment (n = 4), and, for comparison, (— ■ —) rats that received UPC-10 control antibody during the initial collagen challenge and developed arthritis (n = 6).

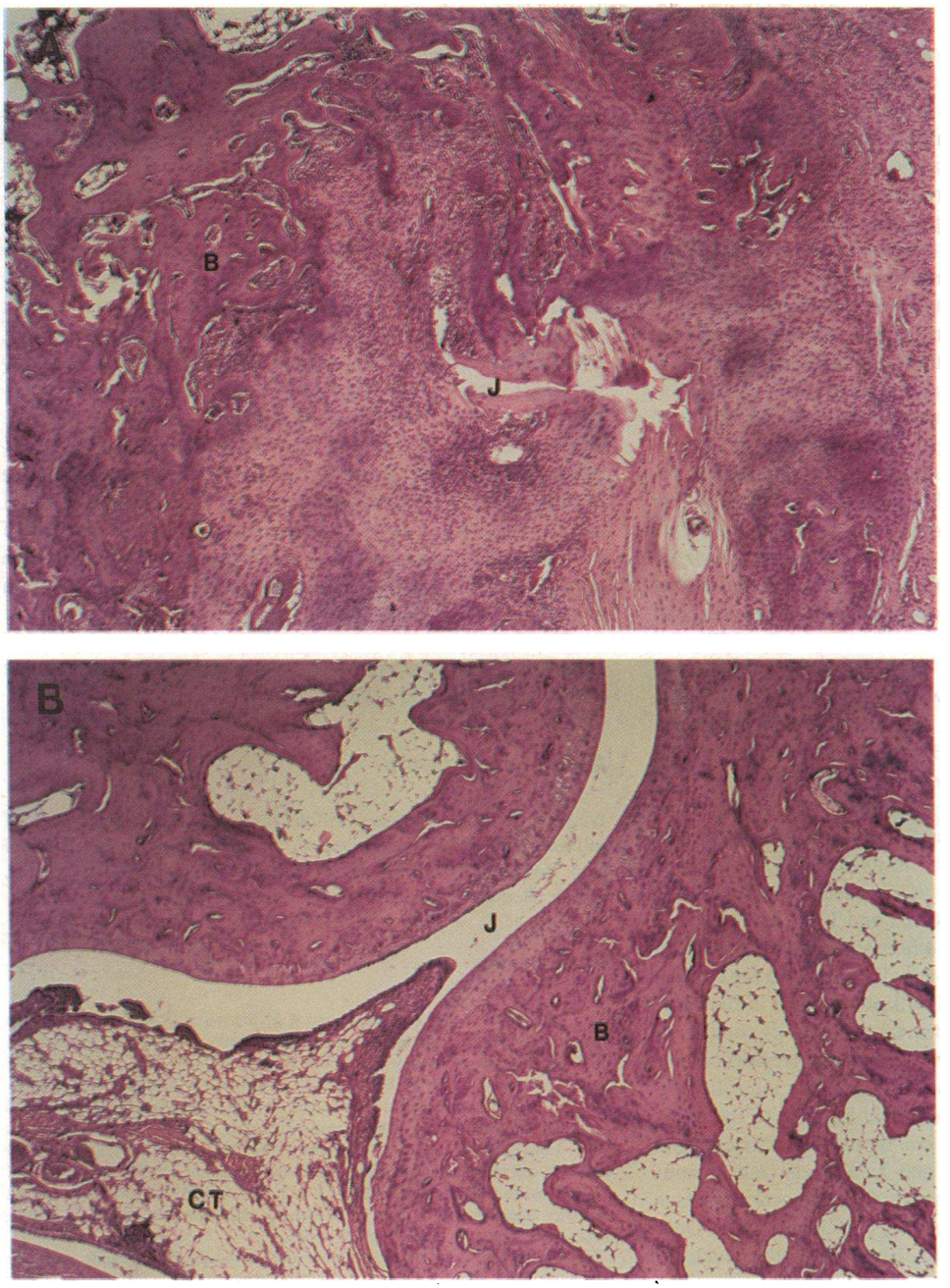

Figure 4. Histological comparison of tibia–tarsal joint sections from BIIC-rechallenged rats. BIIC-immunized, CTLA-4-Ig–treated rats were rechallenged with BIIC at day +50 with concomitant treatment with CTLA-4-Ig, and light micrographs were taken at day +105 of hematoxylin and eosin–stained sections from (*A*) the single rat that developed clinical signs of arthritis (see text), and (*B*) a rechallenged, CTLA-4-Ig–retreated rat that developed no clinical arthritis. B, bone; J, joint space; CT, connective tissue.

rats developed arthritis after collagen rechallenge, with clinical scores of eight and six, respectively, at day +75 and maximum scores of nine and eight during the observation period. The kinetics of arthritis development in these rats were similar to those seen in untreated rats in response to primary collagen immunization (Fig. 1 *A*). Three of the rats treated with CTLA-4-Ig during the rechallenge did not develop arthritis (clinical scores, zero) through the observation period to day +105. One of the CTLA-4-Ig–treated rats developed arthritis by day +61 (clinical score, three), with a maximum score of six at day +105. Anti-BIIC antibody levels remained comparably elevated in the CTLA-4-Ig–treated rat that developed arthritis and in those without arthritis (data not shown). At day +105, histopathology of joints from rats that did and did not develop arthritis after collagen rechallenge was compared (Fig. 4). A hindpaw tibia–tarsal joint from the CTLA-4-Ig–treated rat that developed moderate arthritis following rechallenge shows significant pannus formation and erosion of cartilage and bone (Fig. 4 *A*). By comparison, joints from rechallenged CTLA-4-Ig–treated rats without arthritis showed no histological abnormalities (Fig. 4 *B*).

Discussion

Collagen-induced arthritis in the DR BB/Wor rat is a severe, aggressive disease that affects 100% of immunized animals and proceeds from synovial hypertrophy to pannus formation and articular cartilage and bone destruction within 20 d after heterol-

ogous type II collagen injection (14; Barney Knoerzer, D., B. D. Schwartz, and L. J. Mengle-Gaw, unpublished observations). The histological changes observed during disease progression resemble those seen in rheumatoid arthritis, albeit on a much accelerated temporal scale (Barney Knoerzer, D., and L. Mengle-Gaw, manuscript in preparation); thus collagen-induced arthritis in the DR BB/Wor rat may serve as a useful in vivo disease model for the testing of potential therapeutics relevant to RA. We have shown that treatment with soluble CTLA-4-Ig prevents clinical development of arthritis in the DR BB/Wor rat, when treatment is initiated before collagen immunization. Furthermore, three of four animals receiving a second course of CTLA-4-Ig treatment during rechallenge with collagen were protected from developing clinical signs of arthritis.

Treatment with CTLA-4-Ig between day −1 and day +10 prevented development of clinical manifestations of arthritis (redness, swelling, and digit and ankle deformity) during prolonged observation periods in this model in which 100% of control-treated rats develop persistent arthritis beginning by day +10. Throughout the 50 or more postimmunization days of the observation periods for these experiments, CTLA-4-Ig–treated rats showed no histological abnormalities, with no evidence of the cartilage and bone resorption which completely obliterates the joint architecture of control joints by day +19 (Barney Knoerzer, D., and L. Mengle-Gaw, manuscript in preparation).

Anticollagen antibodies have been implicated in the pathogenesis of disease in the DR BB/Wor rat, as in other rodent collagen arthritis models (20). Arthritic DR BB/Wor rats have high titers of IgG2a anticollagen antibodies (14), and we have shown that passive transfer of serum from arthritic DR BB/Wor rats to naive rats is sufficient to evoke the cartilage and bone erosion associated with full-blown disease (Anderson, G., D. Barney Knoerzer, B. D. Schwartz, and L. J. Mengle-Gaw, unpublished data). By analogy to other rodent collagen-induced arthritis models, these antibodies, initially present in the circulation, may cross-react with type II collagen present in the cartilage of the joint, forming localized antigen–antibody complexes (21, 22). Activated complement components presumably induce the migration of inflammatory cells into the joint. Inflammatory cells are observed at the synovial margins of the joints of BIIC-immunized DR BB/Wor rats by day +9 after collagen injection (Barney Knoerzer, D., and L. Mengle-Gaw, manuscript in preparation). It is unclear whether humoral responses to collagen are involved in the etiology of human RA: Only a small proportion of RA patients have detectable levels of anticollagen antibodies in their sera. However, the majority of RA patients test positive for anticollagen antibodies produced by synovial tissue–derived B cells (23), suggesting that anticollagen autoimmunity may indeed play a role in RA pathogenesis.

Although data from other studies indicate a role for anti-BIIC antibodies in the pathogenesis of collagen-induced arthritis in the BB rat, CTLA-4-Ig–treated rats produced anti-BIIC antibodies but did not develop arthritis. However, the anti-BIIC antibody levels in CTLA-4-Ig–treated rats were consistently lower than in control-treated rats. In other model systems, inhibition or absence of CD28 costimulation has been shown to decrease Th-mediated antibody responses (10, 24) and T cell proliferation (25). The prevention of arthritis by CTLA-4-Ig in our studies suggests that T cell proliferation may be more susceptible to inhibition by CTLA-4-Ig than antibody production and that both T cell proliferation and anti-BIIC antibodies are required to produce arthritis.

In spite of the absence of arthritis, CTLA-4-Ig–treated rats display positive delayed type hypersensitivity responses to bovine IIC at day +28, comparable to those observed in immunized, control-treated animals. This result clearly indicates that CTLA-4-Ig treatment has not caused antigen-specific T cell hyporesponsiveness, or anergy, in these rats. The inability of CTLA-4-Ig–treated rats to generate BIIC-specific DTH responses early in the experimental course may reflect the presence of residual serum CTLA-4-Ig, a hypothesis supported by results of in vivo studies showing slow serum clearance rates for CTLA-4-Ig (10, 26). Prolonged immunosuppression without anergy has been reported in other in vivo experimental systems, where CTLA-4-Ig has been used to block T-dependent antibody responses, and has been suggested to result from variable dependence of certain T cell populations on B7 costimulation (10). Our data indicate that CTLA-4-Ig treatment prevents collagen-induced arthritis and protects rats rechallenged with collagen from developing arthritis, presumably by blocking activation of arthritis-inducing T cells, but does not prevent generation of cells that could participate in a DTH response to collagen. The immunoregulatory network that prevents the in vivo activation of these cells remains to be defined.

The data reported herein suggest that blockade of the CD28/B7 costimulatory pathway may be efficacious for the treatment of human rheumatoid arthritis.

Acknowledgments

We wish to thank Dr. Marie Griffiths for supplying the bovine type II collagen used in these experiments, Dr. Jean Sebaugh for statistical help, Dorothy Edwards for assistance with the histological specimens, and Dr. Bernard Wagner for critical review of the data.

References

1. Mueller, D. L., M. K. Jenkins, and R. H. Schwartz. 1989. Clonal expansion vs functional clonal inactivation: a costimulatory pathway determines the outcome of T cell receptor occupancy. *Annu. Rev. Immunol.* 7:445–480.

2. Jenkins, M. K., D. M. Pardoll, J. Mizuguchi, H. Quill, and R. H. Schwartz. 1987. T cell responsiveness in vivo and in vitro: fine specificity of induction and molecular characterization of the unresponsive state. *Immunol. Rev.* 95:113–135.

3. Linsley, P. S., and J. A. Ledbetter. 1993. The role of the CD28 receptor during T cell responses to antigen. *Annu. Rev. Immunol.* 11:191–212.

4. Linsley, P. S., E. A. Clark, and J. A. Ledbetter. 1990. T cell antigen CD28 mediates adhesion with T cells by interacting with activation antigen B7/BB-1. *Proc. Natl. Acad. Sci. USA.* 87:5031–5035.

5. Linsley, P. S., W. Brady, L. Grosmaire, A. Aruffo, N. K. Damle, and J. A. Ledbetter. 1991. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and IL-2 mRNA accumulation. *J. Exp. Med.* 173:721–730.

6. Gimmi, C. D., G. J. Freeman, J. G. Gribben, K. Sugita, A. S. Freedman, C. Morimoto, and L. M. Nadler. 1991. B cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. *Proc. Natl. Acad. Sci. USA.* 88:6575–6579.

7. Damle, N. K., P. S. Linsley, and J. A. Ledbetter. 1991. Direct helper T cell–induced B cell differentiation invloves interaction between T cell antigen CD28 and B cell activation antigen B7. *Eur. J. Immunol.* 21:1277–1282.

8. Linsley, P. S., W. Brady, M. Urnes, L. S. Grosmaire, N. K. Damle, and J. A. Ledbetter. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med.* 174:561–569.

9. Freeman, G. J., J. G. Gribben, V. A. Boussiotis, J. W. Ng, V. A. Restivo, L. A. Lombard, G. S. Gray, and L. M. Nadler. 1993. Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. *Science (Wash. DC).* 262:909–912.

10. Linsley, P. S., P. M. Wallace, J. Johnson, M. G. Gibson, J. L. Greene, J. A. Ledbetter, C. Singh, and M. A. Tepper. 1992. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. *Science (Wash. DC).* 257:792–795.

11. Lenschow, D. J., Y. Zeng, J. R. Thistlethwaite, A. Montag, W. Brady, M. G. Gibson, P. S. Linsley, and J. A. Bluestone. 1992. Long-term survival of

xenogeneic pancreatic islet grafts induced by CTLA-4-Ig. *Science (Wash. DC).* 257:789–792.

12. Lin, H., S. F. Bolling, P. S. Linsley, R.-Q. Wei, D. Gordon, C. B. Thompson, and L. A. Turka. 1993. Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA-4Ig plus donor-specific transfusion. *J. Exp. Med.* 178:1801–1806.

13. Finck, B. K., P. S. Linsley, and D. Wofsy. 1994. Treatment of murine lupus with CTLA-4Ig. *Science (Wash. DC).* 265:1225–1227.

14. Watson, W. C., J. P. Thompson, K. Terato, M. A. Cremer, and A. H. Kang. 1990. Human HLA-DRβ Gene Hypervariable region homology in the biobreeding BB rat: selection of the diabetic-resistant subline as a rheumatoid arthritis research tool to characterize the immunopathologic response to human type II collagen. *J. Exp. Med.* 172:1331–1339.

15. Schreier, P. H., A. L. M. Bothwell, B. Mueller-Hill, and D. Baltimore. 1981. Multiple differences between the nucleic acid sequences of the $IgG2a^a$ and $IgG2a^b$ alleles of the mouse. *Proc. Natl. Acad. Sci. USA.* 78:4495–4499.

16. Hippenmeyer, P., and M. Highkin. 1993. High level, stable production of recombinant proteins in mammalian cell culture using the herpesvirus VP16 transactivator. *Bio/Technology.* 11:1037–1041.

17. Harper, K., C. Balzano, E. Rouveir, M.-G. Mattei, M.-F. Luciani, and P. Golstein. 1991. Ctla-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *J. Immunol.* 147:1037–1044.

18. Griffiths, M. M., E. J. Eichwald, J. H. Martin, C. B. Smith, and C. W. DeWitt. 1981. Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arthritis & Rheum.* 24:781–789.

19. Luna, L. G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Inc., New York. 258 pp.

20. Myers, L. K., J. M. Seyer, J. M. Stuart, K. Terato, C. S. David, and A. H. Kang. 1993. T cell epitopes of type II collagen that regulate murine collagen-induced arthritis. *J. Immunol.* 151:500–505.

21. Trentham, D. E. 1982. Collagen arthritis as a relevant model for RA. Evidence pro and con. *Arthritis & Rheum.* 25:911–916.

22. Trentham, D. E., and R. A. Dynesius-Trentham. 1989. Type II collagen-induced arthritis in the rat. *In* Pharmacological Methods in the Control of Inflammation. Alan R. Liss, New York. 395–414.

23. Tarkowsky, A., L. Klareskog, H. Carlsten, P. Herberts, and W. J. Koopman. 1989. Secretion of antibodies to types I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis & Rheum.* 32:1087–1092.

24. Ronchese, F., B. Hausmann, S. Hubele, and P. Lane. 1994. Mice transgenic for a soluble form of murine CTLA-4 show enhanced expansion of antigen-specific CD4+ T cells and defective antibody production in vivo. *J. Exp. Med.* 179:809–817.

25. Green, J. M., P. J. Noel, A. I. Sperling, T. L. Walunas, G. S. Gray, J. A. Bluestone, and C. B. Thompson. 1994. Absence of B7-dependent responses in CD28-deficient mice. *Immunity.* 1:501–508.

26. Blazar B. R., P. A. Taylor, P. S. Linsley, and D. A. Vallera. 1994. In vivo blockade of CD28/CTLA-4: B7/BB1 interaction with CTLA-4Ig reduces lethal murine graft-versus-host disease across the major histocompatibility complex barrier in mice. *Blood.* 83:3815–3825.