

Six Month, Double-Blinded, Placebo Controlled,
Dose-Ranging Study (AI-200-004)

Barnett ML, Kremer JM, St. Clair EW, Clegg DO, Furst D,
Weisman M, Fletcher MJF, Lavin PT, Finger E,
Morales A, Le CH, Trentham DE.

Treatment of rheumatoid arthritis with oral type II
collagen: results of a multicenter, double-blind,
placebo controlled trial.

Arthritis and Rheumatism 1998; 41:290-297.

TREATMENT OF RHEUMATOID ARTHRITIS WITH ORAL TYPE II COLLAGEN

Results of a Multicenter, Double-Blind, Placebo-Controlled Trial

MARTHA L. BARNETT, JOEL M. KREMER, E. WILLIAM ST. CLAIR, DANIEL O. CLEGG,
DANIEL FURST, MICHAEL WEISMAN, MALCOLM J. F. FLETCHER, SCOTT CHASAN-TABER,
EDUARDO FINGER, ALEJANDRO MORALES, CHRISTINE H. LE, and DAVID E. TRENTHAM

Objective. Oral administration of cartilage-derived type II collagen (CII) has been shown to ameliorate arthritis in animal models of joint inflammation, and preliminary studies have suggested that this novel therapy is clinically beneficial and safe in patients with rheumatoid arthritis (RA). The present study was undertaken to test the safety and efficacy of 4 different dosages of orally administered CII in patients with RA.

Methods. Two hundred seventy-four patients with active RA were enrolled at 6 different sites and randomized to receive placebo or 1 of 4 dosages (20, 100, 500, or 2,500 $\mu\text{g}/\text{day}$) of oral CII for 24 weeks. Efficacy parameters were assessed monthly. Cumulative response rates (percentage of patients meeting the criteria for response at any time during the study) were analyzed

utilizing 3 sets of composite criteria: the Paulus criteria, the American College of Rheumatology criteria for improvement in RA, and a requirement for $\geq 30\%$ reduction in both swollen and tender joint counts.

Results. Eighty-three percent of patients completed 24 weeks of treatment. Numeric trends in favor of the 20 $\mu\text{g}/\text{day}$ treatment group were seen with all 3 cumulative composite measures. However, a statistically significant increase ($P = 0.035$) in response rate for the 20 $\mu\text{g}/\text{day}$ group versus placebo was detected using only the Paulus criteria. The presence of serum antibodies to CII at baseline was significantly associated with an increased likelihood of responding to treatment. No treatment-related adverse events were detected. The efficacy seen with the lowest dosage is consistent with the findings of animal studies and with known mechanisms of oral tolerance in which lower doses of orally administered autoantigens preferentially induce disease-suppressing regulatory cells.

Conclusion. Positive effects were observed with CII at the lowest dosage tested, and the presence of serum antibodies to CII at baseline may predict response to therapy. No side effects were associated with this novel therapeutic agent. Further controlled studies are required to assess the efficacy of this treatment approach.

Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterized by pain, swelling, and stiffness of multiple joints. Chronic joint inflammation commonly results in progressive joint destruction, deformity, and loss of function. Current therapies for RA are often unsatisfactory, both because of inadequate efficacy and because of unacceptable toxic effects.

Evidence that autoreactive, sensitized T cells

Supported in part by AutoImmune Inc., by grants from the NIH to the General Clinical Research Centers at Beth Israel Hospital (M01-RR-01032), Duke University Medical Center (M01-RR-00030), and University of California, San Diego (M01-RR-00827), and by University of California, San Diego MAC grant 96-6979. Dr. Barnett's salary was supported in part by the Clinical Investigator Training Program, Beth Israel Hospital-Harvard/MIT Health Sciences and Technology, in collaboration with Pfizer Inc.

Martha L. Barnett, MD (current address: University of Virginia Health Sciences Center, Charlottesville), Eduardo Finger, MD, Alejandro Morales, MD, Christine H. Le, MBBS, FRACP, David E. Trentham, MD: Beth Israel Deaconess Medical Center, Boston, Massachusetts; Joel M. Kremer, MD: Albany Medical College, Albany, New York; E. William St. Clair, MD: Duke University Medical Center, Durham, North Carolina; Daniel O. Clegg, MD: University of Utah, Salt Lake City; Daniel Furst, MD: Virginia Mason Research Center, Seattle, Washington; Michael Weisman, MD: University of California, San Diego; Malcolm J. F. Fletcher, MD: AutoImmune Inc., Lexington, Massachusetts; Scott Chasan-Taber, PhD: Boston Biostatistics, Inc., Framingham, Massachusetts.

Address reprint requests to David E. Trentham, MD, Division of Rheumatology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215.

Submitted for publication January 16, 1997; accepted in revised form September 19, 1997.

participate in sustaining the inflammation of RA (1) provides direction to the search for therapeutic strategies based on specific immunosuppressive actions that would be both highly effective and minimally toxic. The ability to induce antigen-specific peripheral immune tolerance by oral administration of antigens has been recognized for some time (2), and it is presumed that the physiologic interaction of proteins with the gut immune system has evolved to prevent systemic immune responses to ingested proteins. The possibility that autoimmune disease may be ameliorated by the induction of oral tolerance to disease-relevant autoantigens is currently under active investigation. It has been shown that oral administration of autoantigens suppresses a variety of experimental autoimmune diseases, including experimental allergic encephalomyelitis (EAE) (3,4), as well as collagen- (5,6), adjuvant- (7), and antigen- (8) induced arthritis. Preliminary results in humans have been encouraging, primarily in patients with multiple sclerosis using peroral administration of myelin (9) and in patients with RA using peroral administration of type II collagen (CII) (10,11).

Although the precise mechanisms of oral tolerance are not fully known, studies of EAE have shown, by immunohistologic analysis of brain tissue, that oral antigen delivery preferentially increases expression of the inhibitory cytokines transforming growth factor β (TGF β) and interleukin-4 (IL-4), and decreases expression of the proinflammatory cytokines IL-1, IL-2, IL-6, IL-8, tumor necrosis factor α (TNF α), and interferon- γ (12). The net outcome is a lessening of brain inflammation. Given in low doses, orally administered antigens induce active immune suppression, whereas high antigen doses lead to clonal anergy (13) or deletion (14).

In addition to its positive effect in animal models of arthritis, oral administration of CII may benefit humans with RA. A placebo-controlled, phase II study involving 60 adults with severe, active RA demonstrated significant ($P < 0.03$) decreases in tender and swollen joint counts after 3 months of oral CII treatment (10). A 3-month open-label trial of CII in juvenile rheumatoid arthritis also was associated with clinical improvement in disease measures without significant toxicity (15). The present multicenter, double-blind, dose-ranging study of oral CII in adult RA was undertaken as a phase II trial to extend these earlier results and to investigate the relationship between dosage and clinical response.

PATIENTS AND METHODS

Patients. To be eligible for this trial, patients had to meet the 1987 American College of Rheumatology (ACR;

formerly, the American Rheumatism Association) classification criteria for RA (16) and be between the ages of 18 and 80 years, with onset of RA after age 16. Patients were required to have at least 6 swollen and 9 tender joints, and duration of disease had to have been at least 6 months. Women of childbearing potential were required to have a negative pregnancy test result at baseline and to be using an effective contraceptive measure. Patients were required to discontinue treatment with disease-modifying antirheumatic drugs (DMARDs) before entering the study, with a variable washout period depending on the specific medication (at least 8 weeks for methotrexate, azathioprine, minocycline, cyclosporine, and cyclophosphamide, and at least 12 weeks for hydroxychloroquine, sulfasalazine, oral or intramuscular gold, and penicillamine).

Patients were excluded from the trial if they had previously been treated with oral CII or anti-TNF antibodies. Patients taking therapeutic doses of fish oil or plant oil (≥ 2 gm/day), or shark cartilage or cartilage from another species were also excluded. Patients were not eligible if they had documented human immunodeficiency virus infection, a history of substance abuse within 1 year prior to study entry, a history of gastrointestinal disease which might affect collagen absorption and processing, serious intercurrent/underlying disease which would limit successful participation in the trial, or were classified in Steinbrocker functional class IV (17).

Study design. The study was a 6-center, double-blind, randomized, placebo-controlled trial comparing different dosages of CII and placebo in the treatment of RA. Upon meeting all entry criteria, patients were enrolled and were subsequently stratified by rheumatoid factor status. This was done to ensure that the treatment groups would be balanced with regard to rheumatoid factor positivity. At each of the 6 investigative sites, randomization of patients to the 5 treatment groups was accomplished in blocks of 5 within each stratum (positive versus negative for rheumatoid factor). Patients and investigators were blinded to the treatment regimens throughout the study.

Patients were allowed to remain on their nonsteroidal antiinflammatory drug (NSAID) and/or oral corticosteroid regimens provided that the steroid dosage was ≤ 10 mg of prednisone equivalent, and that neither the NSAID nor steroid dosage was changed during the study. Patients who requested analgesics for pain management were supplied these medications as needed during the period of study. Analgesic ingestion was recorded at each visit.

Patients were provided with a 1-month supply of masked study medication at the baseline visit and every 4 weeks thereafter. Liquid study medication was supplied in the form of patient kits containing each daily dose in individual polystyrene tubes. The study medication required refrigeration. Therefore, patients were given coolers to transport the medication home and were told to keep the kits in the refrigerator. Patients were instructed to empty the contents of one tube into a 4–6-ounce glass of cold orange juice 20 minutes prior to eating breakfast, and were required to consume the entire amount every morning.

Study medication. Native CII was prepared from the sternal cartilage of chickens by the method of Trentham et al (18). The CII was diluted in 0.1M acetic acid, and supplied as a single daily dose of 20 μ g, 100 μ g, 500 μ g, or 2,500 μ g. The

placebo was an equivalent volume of dilute acetic acid that was visually indistinguishable from the active drug.

Outcome measures. Clinical assessments of efficacy were made at baseline and repeated 2, 4, 8, 12, 16, 20, and 24 weeks later (except the Health Assessment Questionnaire [HAQ] [19], which was completed only at baseline and weeks 12 and 24). Fifty-four diarthrodial joints were examined for the presence or absence of tenderness, and 52 were examined for swelling (hips excluded). Joint counts for tenderness and swelling were the sum of the number of affected joints (20). These joints were also evaluated for the severity of both tenderness and swelling using a 4-point scale, in which 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The sum of these determinations for all joints constituted a collective score for either tenderness or swelling (20). During the trial, a strong emphasis was placed on having all joint assessments for each patient done by the same evaluator.

Grip strengths were measured bilaterally using mercury-strain sphygmomanometers which were fitted with custom-made covers to assure a uniform circumference of the cuff. Fifty-foot walking times were measured in seconds. Physician and patient global assessments of disease activity were performed using a scale of 1-5, in which 1 = absent, 2 = mild, 3 = moderate, 4 = severe, and 5 = very severe. In addition, both the physicians and the patients rated progress from the previous visit, according to the categories much worse, worse, same, better, or much better (20). Patients were questioned about the duration of morning stiffness experienced on the day before each study visit. Functional class was assessed according to the Steinbrocker classification (17) at the screening visit and again at 24 weeks. Functional status was assessed at baseline and at weeks 12 and 24 using the HAQ.

Adverse events were monitored at every visit. Moreover, at baseline and at weeks 2, 4, 12, and 24, laboratory parameters were measured to assess safety. The erythrocyte sedimentation rate (ESR) was obtained by the Westergren method at baseline and at every visit thereafter, and rheumatoid factor positivity was determined at the screening visit and at week 24. Extra serum samples obtained at baseline, week 12, and week 24 were frozen and stored until the end of the study, for measurement of C-reactive protein (CRP) and IgA and IgG antibody titers to native chicken CII by enzyme-linked immunosorbent assay (ELISA). The ELISA was performed utilizing wells coated with purified CII, patients' serum, and horseradish peroxidase-linked goat anti-human IgA and IgG antibodies.

Patients who dropped out before completing 24 weeks of treatment were evaluated immediately prior to exiting the study. During this early-termination visit, safety and efficacy measurements were obtained.

Statistical analysis. Safety assessments were performed on all patients who consumed any masked study medication. Efficacy analyses were performed on the intent-to-treat population (all patients for whom we had at least 1 post-baseline measurement) as well as on the population of patients who completed the 24-week study. Balance within each treatment group with respect to patient characteristics at baseline was assessed, using Fisher's exact test for categorical variables and analysis of variance for continuous variables. Differences were considered statistically significant if the 2-sided *P* value was ≤ 0.05 .

The primary efficacy end point was cumulative response, paralleling other recent studies of potential therapies for RA (21,22). Patients were said to be "responders" if they met the response criteria at any time during the study and were said to be "nonresponders" if they never showed a response to treatment during the study. This approach allowed each patient to be assessed just once, thus avoiding the criticisms of repeated significance testing and dropout effects, and was thought to be appropriate for a phase II trial which was primarily exploratory in nature. Response was analyzed with respect to 3 composite response-criteria sets. These were the Paulus criteria (23), the ACR criteria for improvement in RA (24), and the requirement for an improvement of $\geq 30\%$ in both the tender and the swollen joint counts. The a priori contrasts planned for this study were to compare each active treatment group with the control group. The study had 80% power to detect a 25% difference in response rates between best dose and placebo (which was expected to have a response rate of 20%) for a 2-sided hypothesis test with 5% type I error. A logistic regression model enabled us to simultaneously compare response rates between each active treatment and placebo group while controlling for patient characteristics, without making multiple comparisons. The patient characteristics included in the model were age, sex, baseline tender and swollen joint counts, baseline rheumatoid factor status and ESR, Steinbrocker functional class at baseline, DMARD wash-out interval prior to baseline, baseline NSAID use, baseline corticosteroid use, duration of RA, the extent of analgesic use during the study, and study site. Interactions between each treatment level and each of the covariates were assessed.

Individual disease parameters that were analyzed as primary efficacy end points included tender and swollen joint counts, and physician and patient global assessments of disease severity. Safety was assessed by comparing the type and incidence of treatment-emergent events and laboratory abnormalities reported. Adverse events were classified using COSTART.

RESULTS

A total of 297 patients with active RA were screened for study eligibility, of whom 274 met the entrance criteria and were randomized to receive 1 of 4 doses of CII or matching placebo for a 24-week period. The characteristics of the enrolled patients are summarized in Table 1. There were no statistically significant differences between the treatment groups in demographic or disease characteristics. One patient in the 500 $\mu\text{g}/\text{day}$ CII group dropped out prior to receiving any post-baseline efficacy assessments and was therefore not included in the intent-to-treat analysis.

A total of 228 patients (83%) completed the full 6-month treatment period. The frequency of dropouts was similar across all treatment groups. Of the 46 dropouts, 42 were due to inadequate therapeutic effect

Table 1. Patient characteristics at baseline, by treatment group*

Variable	Placebo (n = 57)	Oral type II collagen			
		20 µg/day (n = 54)	100 µg/day (n = 55)	500 µg/day (n = 53)	2,500 µg/day (n = 54)
Age, years	53.6 ± 12.1	53.5 ± 11.3	50.3 ± 10.9	49.4 ± 13.0	51.3 ± 14.1
Sex, % female	89	76	89	81	83
Disease duration, years	13.1 ± 9.6	12.8 ± 8.1	11.7 ± 8.4	9.8 ± 8.3	11.8 ± 10.9
RF positive, %	72	76	76	75	74
Steinbrocker functional class, %					
Class I	0	2	2	4	2
Class II	81	74	78	85	80
Class III	19	24	20	11	18
Westergren ESR, mm/hour	36 ± 22	41 ± 32	36 ± 27	35 ± 23	38 ± 26
NSAID use at entry, %	81	76	87	79	78
Oral corticosteroid use at entry, %	53	59	47	55	37
Previous DMARD use, %	89	87	91	96	81
No. of dropouts during study	12	11	5	7	10

* Except where otherwise indicated, values are the mean ± SD. There were no statistically significant differences in any feature between the treatment groups. RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; NSAID = nonsteroidal antiinflammatory drug; DMARD = disease-modifying antirheumatic drug.

and 4 were due to adverse events (1 from the placebo group and 3 from the CII group). Although some of the patients took additional antiinflammatory medications during the trial, none were dropped from the analysis because of these protocol violations. Rules were developed before the analysis to address the use of protocol-excluded medical therapy. If a patient received an intraarticular corticosteroid injection, that joint was automatically assigned a grade of 3 (severe) for both tenderness and swelling at the next visit. This convention affected efficacy data for a single joint at 1 visit in 7 patients. Because of concern about possible systemic effects from an intraarticular steroid injection, a review of clinical efficacy data was undertaken to determine whether any of these 7 patients met the criteria for response only at the visit immediately following a joint injection. This was determined not to be the case for any of these 7 patients. If a patient took any antiinflammatory drug prohibited by the protocol for 2 of the 3 days prior to a visit, efficacy data from that visit were excluded from all analyses. The application of this rule did not result in the exclusion of any efficacy data.

Clinical response was assessed in the intent-to-treat population with respect to 3 composite response indices: the Paulus criteria, the ACR criteria for improvement in RA, and a reduction of ≥30% in both tender and swollen joint counts. In the 20 µg/day treatment group, this analysis revealed statistically significant improvement in the percentage of patients who met the cumulative Paulus criteria during the study (21 of 54, or 39%) versus the placebo group (11 of 57, or 19%; $P = 0.035$ by Fisher's exact test). This difference

remained significant when potential confounding factors (investigative site, baseline use of NSAIDs or steroids, rheumatoid factor positivity, functional class, baseline joint counts, ESR, age, sex, duration of disease, and analgesic use) were accounted for in a logistic regression model ($P = 0.017$ by Wald chi-square test) (25). The percentage of responders (according to the Paulus criteria) in the other 3 treatment groups decreased incrementally with each increase in dosage of CII (Figure 1). No statistically significant interactions between the treatment and any of the covariates were observed.

Response rates for each of the 3 composite

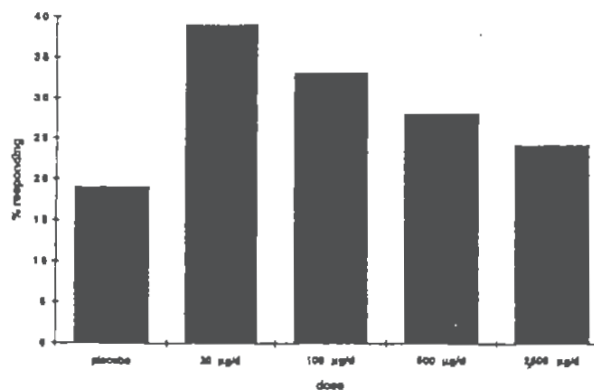


Figure 1. Percentage of patients with rheumatoid arthritis, in the intent-to-treat analysis, meeting the Paulus criteria for response to treatment with type II collagen (CII) versus placebo at any time during the study. The difference in response rates for placebo versus 20 µg/day of CII was statistically significant ($P < 0.05$).

Table 2. Cumulative response rates for composite response criteria, by treatment group*

Composite measure	Placebo	Oral type II collagen			
		20 $\mu\text{g/day}$	100 $\mu\text{g/day}$	500 $\mu\text{g/day}$	2,500 $\mu\text{g/day}$
Paulus criteria	19.3	38.9†	32.7	28.3	24.1
ACR criteria	17.5	25.9‡	20.0	24.5	22.2
$\geq 30\%$ criteria§	31.6	46.3¶	40.0	43.4	40.7

* Values are percentages. ACR = American College of Rheumatology.
 † $P = 0.035$ vs. placebo.
 ‡ $P = 0.360$ vs. placebo.
 § $\geq 30\%$ reduction in both swollen joint count and tender joint count.
 ¶ $P = 0.123$ vs. placebo.

criteria sets are shown in Table 2. Following the same statistical analysis plan, numeric trends in favor of the 20 $\mu\text{g/day}$ group were seen using the ACR criteria and the $\geq 30\%$ reduction criteria, although there was no clear difference in response rates between the doses used. Since followup evaluations of functional status were

conducted only at weeks 12 and 24, when the HAQ was administered, it is important to note that the ACR criteria were therefore assessed at fewer time points than the other criteria. The inclusion of functional status improvement as a feature of the ACR criteria thus resulted in lower absolute numeric values for responders in this study.

The 4 individual measures selected as primary efficacy end points were swollen and tender joint counts, and physician and patient global assessments of disease activity. Changes in these parameters among the treatment groups during the study are shown in Figures 2A–D. Numeric trends in favor of the 20 $\mu\text{g/day}$ group versus the other treatment groups were seen for 3 of the 4 variables (tender joint count, and physician and patient global scores), although there were no statistically significant differences between the treatment groups at 24 weeks.

There were no statistically or clinically significant changes from baseline, or differences between treatment groups, in any of the measured blood or urine safety

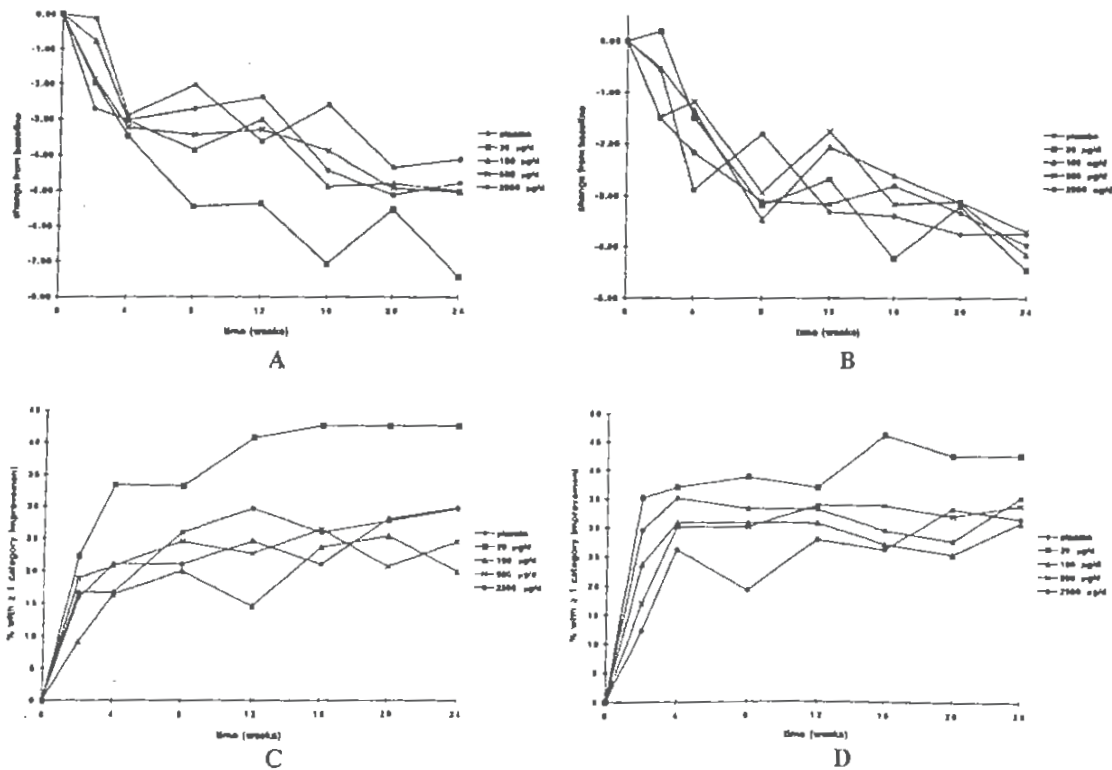


Figure 2. Changes in individual efficacy variables from baseline to 24 weeks by treatment group (intent-to-treat population). A, Tender joint count. B, Swollen joint count. C, Percentage improvement in the physician global score for disease activity. D, Percentage improvement in the patient global score for disease activity.

parameters. A review of individual patient values at baseline revealed hematologic abnormalities typical of patients with RA. Serum and urine chemistry results were unremarkable during the study, except in 6 patients who developed transaminase elevations greater than twice the upper limit of the reference range. These 6 patients included 1 in the placebo group, 1 in the 20 $\mu\text{g}/\text{day}$ treatment group, 1 in the 100 $\mu\text{g}/\text{day}$ group, and 3 in the 2,500 $\mu\text{g}/\text{day}$ group. The highest transaminase level recorded was an alanine aminotransferase value of 583 IU. Three of the 6 patients stopped taking the study medication, resulting in normalization of their transaminase levels, which was followed by a rechallenge with CII and no subsequent liver enzyme abnormalities. The other 3 patients also discontinued the study medication, with subsequent normalization of their transaminase levels; these 3 patients were not rechallenged with CII. Overall, there were no differences between the treatment groups in the incidence of any adverse event during this study (data available on request), and no serious adverse event was attributed to CII.

At baseline, serum IgA and IgG antibodies to CII were detected in 37% and 18% of patients, respectively. When the aggregate group of all patients treated with any dosage of CII was compared with the total placebo group, the presence of anticollagen antibodies was significantly associated with an increased likelihood of achieving a clinical response to CII as defined by the Paulus criteria. Among patients with IgA antibodies present at baseline, 28 (39.4%) of 71 CII-treated patients responded, while only 4 (13.8%) of 29 patients in the placebo group responded. Among patients with IgG antibodies at baseline, 15 (45.5%) of 33 CII-treated patients responded as compared with 2 (13.3%) of 15 patients in the placebo group. These differences were statistically significant ($P < 0.02$ for IgA positivity and $P < 0.05$ for IgG positivity, by Fisher's exact test). There was no significant change in IgA or IgG antibody status with treatment, suggesting that sensitization to CII did not occur. Levels of CRP at baseline did not correlate statistically with the likelihood of response, nor did they change significantly with therapy.

DISCUSSION

We report herein the results of the first multicenter study of oral tolerance in the treatment of human autoimmune disease. Utilizing the cumulative Paulus criteria for response, CII at a dosage of 20 $\mu\text{g}/\text{day}$ was shown to be superior to placebo in the treatment of RA. Although there was no clear difference between the

dosages used, numeric trends in favor of the 20 $\mu\text{g}/\text{day}$ dosage were also seen using the 2 other composite criteria sets, as well as using 3 of the 4 primary individual efficacy parameters that were followed up. As a group, the patients studied in this trial had severe arthritis, with a mean \pm SD duration of disease of 11.2 ± 9.1 years. At study entry, patients had a mean \pm SD of 24 ± 11 swollen joints and 27 ± 11 tender joints, and 85% of the patients had taken DMARDs previously. While statistically significant improvement was demonstrated with only 1 of the 3 composite criteria sets applied, the mild antirheumatic properties of CII observed in this study must be interpreted in the context of the disease duration and severity in the cohort. Furthermore, CII was shown to have an excellent safety profile that was clinically and immunologically indistinguishable from that of placebo. The high completion rate of 83% of patients enrolled further attests to the tolerability of CII.

A previous study by Trentham et al (10) revealed a positive clinical effect in patients treated with CII at a dosage of 100 $\mu\text{g}/\text{day}$ for the first month and 500 $\mu\text{g}/\text{day}$ for months 2 and 3. The dose range in the present study was chosen to incorporate these previously studied dosages, in addition to both a lower and a higher dosage, to try to elucidate the most effective level of treatment. The clinical effects seen at the 100 $\mu\text{g}/\text{day}$ and 500 $\mu\text{g}/\text{day}$ dosages in the present study were not as great as those reported in the previous dose-escalation study (10), but the patients in that trial had less severe disease at baseline and no DMARD washout was required, both of which may have contributed to this observed difference. The recent study of oral type II collagen in the treatment of RA by Sieper et al (11) revealed a higher prevalence of responders among collagen-treated patients versus those receiving placebo, although statistically significant differences between treatment groups were not found. In the study by Sieper et al, bovine, rather than chicken, collagen was used, and the dosages given (1 mg/day and 10 mg/day) were higher than those utilized in the present study. Nonetheless, their finding of a good response in a subset of patients is intriguing, and it leads one to speculate that an immunologic difference might exist that could render some patients more likely to respond to this particular form of therapy.

This study has several limitations. The use of 3 composite criteria sets as end points makes it more difficult to draw conclusions about the clinical efficacy of CII. Given that a statistically significant effect was demonstrated with only 1 of the 3 composite criteria sets tested, it would be premature to interpret these results as conclusive evidence of efficacy. However, since this was a phase II trial, a

primary objective was to gather as much information as possible about effectiveness, toxicity, and optimal dose. Analyzing response rates using a cumulative-response standard can also be criticized, because patients who simply exhibit oscillations in their disease activity (rather than true response to treatment) might be counted as responders. Although this is a valid concern, response rates were compared with those in a placebo group of patients who had an equal likelihood of similar disease fluctuations. Because patients were divided among 5 different treatment groups, the numbers of patients in each group were relatively small, particularly for a multicenter trial. In addition, patients in this study tended to have severe disease of long duration, and therefore, no conclusions can be reached about the use of CII to treat patients with milder or early-stage RA. The dosages of CII tested in this study may not have been optimal, given that the lowest dosage showed the greatest efficacy. Ideally, dosages both lower and higher than the most effective dosage should have been included. Finally, the format of this trial precluded knowing whether the same dosage of collagen is best for all patients.

Our finding that the 20 $\mu\text{g}/\text{day}$ dosage proved to be most effective, and that the response rates decreased with each successive increase in dosage, is consistent with observations in animal studies. Active suppression of inflammation occurs in animal models with lower doses of oral tolerogen, but not with higher doses at which anergy and clonal deletion of T cells reactive against the fed antigen are seen (13,14). In a rat model of adjuvant arthritis, the dosage of oral CII most effective in suppressing disease was 3 $\mu\text{g}/\text{day}$ (7). The mechanism of oral tolerance is believed to involve the generation of an immune response in the gut, in a manner analogous to oral vaccinations. Given that dosages of oral vaccines are not routinely adjusted based on body weight, the efficacy seen with the lowest dosage tested in the present study is believed to be consistent with the animal data.

Orally dosed exogenous antigen has recently been reported to activate CD4+ and noncytotoxic CD8+ regulatory T cells that may contribute to a down-regulation of cellular and humoral immunity (26,27). The regulatory cells that orchestrate active suppression appear to act via the secretion of inhibitory cytokines, such as TGF β and IL-4 (12,27). It is presumed that active suppression of inflammation by these regulatory lymphocytes requires migration of these cells to a local microenvironment, which contains a protein resembling the orally dosed heterologous antigen. Because the regulatory cells generated by oral tolerization are

primed in an antigen-specific manner but suppress in an antigen-nonspecific manner, they mediate "bystander suppression" when they encounter the orally dosed autoantigen at sites of inflammation (28,29). Thus, it may not be obligatory to identify the target autoantigen for a given disease. What is required is to orally administer a protein that is present at the site of inflammation and is capable of inducing regulatory cells to secrete suppressive cytokines. These findings have important implications for the use of oral tolerance as a therapeutic approach for the treatment of T cell-mediated inflammatory autoimmune diseases in humans, in which the inciting autoantigen is unknown or in which there is autoreactivity to multiple autoantigens in the target tissue. The results in the present study, with maximal efficacy being demonstrated with the lowest dosage of CII tested, are consistent with the results of animal trials (7,8) and with the hypothesis that the mechanism of action of oral tolerance in this setting is active suppression of inflammation rather than clonal anergy or deletion. This finding also suggests that CII may be a useful therapeutic protein even if type II collagen is not of major pathogenetic importance as an autoantigen in established RA or is only one of multiple autoantigens that are involved.

It is of interest that the presence at baseline of serum antibodies to CII, of either the IgA or IgG isotype, was associated with an increased probability of achieving a clinical response after treatment with CII. The basis for this association remains to be explained. Bystander suppression would be expected to benefit patients regardless of whether autoreactivity to CII is involved in the pathogenesis of RA. It is possible that there is a subset of patients with RA in which autoimmunity to CII is of pathogenic importance. It recently has been reported that the presence of antibodies to CII in patients with early RA may be predictive of rapidly progressive disease (30). In patients with antibodies to CII, oral dosing with CII might trigger antigen-specific mechanisms such as anergy or deletion. Alternatively, it is possible that patients with serum antibodies to CII are more likely to generate regulatory cells which would mediate bystander suppression after oral dosing with CII. Further research is needed to confirm this association and to determine whether screening for collagen antibodies would be warranted in deciding which patients to treat with CII.

The phenomenon of oral tolerance represents a novel approach to the treatment of chronic, disabling autoimmune diseases. To date, no serious adverse events have been noted in animal or human studies of

oral tolerance, and the simplicity and apparent safety of this form of treatment make it extremely appealing. Although efficacy of oral CII at 20 $\mu\text{g}/\text{day}$ was found to be statistically significant with only 1 of the 3 composite criteria tested, the excellent safety profile and suggestion of efficacy indicate that additional clinical investigations with this protein are warranted, particularly in patients whose disease has not reached the stage and severity encountered in this trial. These studies should also include further analysis of potential immunologic predictors of response to CII.

ACKNOWLEDGMENTS

The authors thank their patient volunteers for participating in the study, and acknowledge the following individuals for their invaluable contributions as study coordinators: Rose-lynn Dynesius-Trentham (Beth Israel Hospital), Mari Kaymakejian (Albany Medical College), Edna Scarlett and Dr. Al Bello (rheumatology fellow), who helped with the conduct of the study (Duke University Medical Center), Sidney D. Hughes (University of Utah), E. Ching Voon (Virginia Mason Medical Center), and Denise Mathes (University of California, San Diego). The authors are also grateful for the statistical expertise of John Balsler, PhD, in study design and Philip T. Lavin, PhD, in data analysis (Boston Biostatistics, Inc.).

REFERENCES

- Sewell KL, Trentham DE. Pathogenesis of rheumatoid arthritis. *Lancet* 1993;341:283-6.
- Mowat A. The regulation of the immune responses to dietary protein antigens. *Immunol Today* 1987;8:93-8.
- Higgins PJ, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. *J Immunol* 1988;140:440-5.
- Bitar DM, Whitacre CC. Suppression of experimental autoimmune encephalomyelitis by the oral administration of myelin basic protein. *Cell Immunol* 1988;112:364-70.
- Nagler-Anderson C, Bobber LA, Robinson ME, Siskind GW, Thorbecke GJ. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc Natl Acad Sci U S A* 1986;83:7443-6.
- Thompson HSG, Staines NA. Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritis in rats. *Clin Exp Immunol* 1986;64:581-6.
- Zhang ZY, Lee CS, Lider O, Weiner HL. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. *J Immunol* 1990;145:2489-93.
- Yoshino S, Quattrocchi E, Weiner HL. Suppression of antigen-induced arthritis in Lewis rats by oral administration of type II collagen. *Arthritis Rheum* 1995;38:1092-6.
- Weiner HL, Mackin GA, Matsui M, Orav EJ, Khoury SJ, Dawson DM. Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science* 1993;259:1321-4.
- Trentham DE, Dynesius-Trentham RA, Orav EJ, Combitchi D, Lorenzo C, Sewell KL, et al. Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* 1993;261:1727-30.
- Sieper J, Kary S, Sørensen H, Alten R, Eggens U, Hüge W, et al. Oral type II collagen treatment in early rheumatoid arthritis: a double-blind, placebo-controlled, randomized trial. *Arthritis Rheum* 1996;39:41-51.
- Khoury SJ, Hancock WW, Weiner HL. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor β , interleukin 4, and prostaglandin E expression in the brain. *J Exp Med* 1992;176:1355-64.
- Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci U S A* 1994;91:6688-92.
- Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995;376:177-80.
- Barnett ML, Combitchi D, Trentham DE. A pilot trial of oral type II collagen in the treatment of juvenile rheumatoid arthritis. *Arthritis Rheum* 1996;39:623-8.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
- Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. *JAMA* 1994;271:659-62.
- Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen: an experimental model of arthritis. *J Exp Med* 1977;146:857-68.
- Fries JF, Spitz PW, Young DY. The dimensions of health outcomes: the health assessment questionnaire, disability and pain scales. *J Rheumatol* 1982;9:789-93.
- Weinblatt ME, Coblyn JS, Fox DA, Fraser PA, Holdsworth DE, Glass DN, et al. Efficacy of low-dose methotrexate in rheumatoid arthritis. *N Engl J Med* 1985;312:818-22.
- Matteson EL, Yocum DE, St. Clair EW, Achkar AA, Thakor MS, Jacobs MR, et al. Treatment of active refractory rheumatoid arthritis with humanized monoclonal antibody CAMPATH-1H administered by daily subcutaneous injection. *Arthritis Rheum* 1995;38:1187-93.
- Van der Lubbe PA, Dijkmans BAC, Markkuse HM, Næssander U, Breedveld FC. A randomized, double-blind, placebo-controlled study of CD4 monoclonal antibody therapy in early rheumatoid arthritis. *Arthritis Rheum* 1995;38:1097-106.
- Paulus HE, Egger MJ, Ward JR, Williams HJ, and The Cooperative Systematic Studies of Rheumatic Diseases Group. Analysis of improvement in individual rheumatoid arthritis patients treated with disease-modifying antirheumatic drugs, based on the findings in patients treated with placebo. *Arthritis Rheum* 1990;33:477-84.
- Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995;38:727-35.
- Cox DR. The analysis of binary data. London: Methuen; 1970.
- Ke Y, Kapp JA. Oral antigen inhibits priming of CD8+ CTL, CD4+ T cells, and antibody responses while activating CD8+ suppressor T cells. *J Immunol* 1996;156:916-21.
- Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994;265:1237-40.
- Miller A, Lider O, Weiner HL. Antigen-driven bystander suppression after oral administration of antigens. *J Exp Med* 1991;174:791-8.
- Al-Sabbagh A, Miller A, Santos LMB, Weiner HL. Antigen-driven tissue-specific suppression following oral tolerance: orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mouse. *Eur J Immunol* 1994;24:2104-9.
- Cook AD, Rowley MJ, Mackay IR, Gough A, Emery P. Antibodies to type II collagen in early rheumatoid arthritis: correlation with disease progression. *Arthritis Rheum* 1996;39:1720-7.