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ORAL TYPE II COLLAGEN TREATMENT IN EARLY RHEUMATOID ARTHRITIS

A Double-Blind, Placebo-Controlled, Randomized Trial

JOACHIM SIEPER, SONJA KARY, HELMUT SÖRENSEN, RIEKE ALTEN, ULRICH EGGENS, WERNER HÜGE, FALK HIEPE, ANDREA KÜHNE, JOACHIM LISTING, NORBERT ULBRICH, JÜRGEN BRAUN, ANGELA ZINK, and NICHOLAS AVRION MITCHISON

Objective. **To investigate the efficacy of oral type II collagen in the treatment of early rheumatoid arthritis (RA).**

Methods. **Ninety patients with RA (disease duration ≤3 years) were treated for 12 weeks with oral bovine type II collagen at 1 mg/day (n = 30) or 10 mg/day (n = 30) or with placebo (n = 30), in a double-blind randomized study.**

Results. **There was no significant difference between the 3 groups in terms of response to treatment. However, we observed a higher prevalence of responders in the type II collagen–treated groups: 7 responders in the 10-mg type II collagen group and 6 in the 1-mg group, versus 4 in the placebo group. Furthermore, 3 patients in the 10-mg type II collagen group and 1 patient in the 1-mg type II collagen group, but no patients in the placebo group, had very good response. A total of 14 patients had to be withdrawn from the study: 2 because of side effects (nausea) and 12 because of lack of efficacy.**

Conclusion. **Only a minority of patients responded to treatment with oral type II collagen. These results justify further efforts to identify which patients will have a good response to such therapy.**

Oral tolerance therapy has long been recognized as being able to induce peripheral immune tolerance to specific antigen (1,2). Low doses of orally administered antigen favor active suppression of T cell–mediated immune responses, whereas high doses can induce peripheral tolerance. Treatment with orally fed antigens has proved highly effective in various animal models of human autoimmune diseases, including collagen-induced arthritis (3) and adjuvant arthritis (4), two models that resemble human rheumatoid arthritis (RA). Type II collagen has been selected for use in oral tolerance trials in RA because of its restricted location in cartilage (and the eye) and its abundance (5,6). There is no convincing evidence that collagen itself drives the disease (7,8).

Antigen-specific bystander suppression has been described in several animal models as a mechanism of oral tolerance, where the antigen used is not responsible for the chronic immune response in the target organ (4,9). Thus, in the case of RA, T cells primed to type II collagen in the gut would release suppressive cytokines after a second stimulation by type II collagen in the inflamed joint. From animal experiments, there is evidence that the peripheral suppression is mediated by Th2 cytokines such as interleukin-4 (IL-4), IL-10, and transforming growth factor β (TGFβ), secreted by T cells that are specifically activated in the Peyer's patches of the gut (10). Thus, oral tolerance can be regarded as one approach to rectifying imbalance in T cell cytokine levels.

Although there has been debate in the past

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Joachim Sieper, MD: Deutsches Rheuma Forschungszentrum, Berlin, and Klinikum Benjamin Franklin, Free University, Berlin, Germany; Sonja Kary, MD, Andrea Kühne, Joachim Listing, PhD, Norbert Ulbrich, PhD, Angela Zink, PhD, Nicholas Avrion Mitchison, PhD: Deutsches Rheuma Forschungszentrum, Berlin; Helmut Sörensen, MD: Immanuel Hospital, Berlin; Rieke Alten, MD: Schlossparkklinik, Berlin; Ulrich Eggens, MD, Jürgen Braun, MD: Klinikum Benjamin Franklin, Free University, Berlin; Werner Hüge, MD: Rheumaklinik Buch, Berlin; Falk Hiepe, MD: Humboldt University, Berlin.

Address reprint requests to Joachim Sieper, MD, Department of Medicine, Rheumatology, Klinikum Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany.

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about the role of T cells in the pathogenesis of RA (11,12), there is now increasing evidence of their importance. Several investigators have been able to detect T cell cytokines in RA synovial membrane by molecular genetic (13) or immunohistologic methods (14,15). Furthermore, there is evidence that the Th1 cytokine interferon-γ (IFNγ) predominates in RA synovial membrane (13), as it does in animal models of other T cell–mediated autoimmune diseases such as multiple sclerosis (16) and diabetes mellitus (17). Thus, the concept has arisen that Th1 cells secreting mainly IFNγ are responsible for the chronic immune response against an unknown self antigen, while Th2 cytokines such as IL-4 and IL-10 would inhibit such a immune response (18). This provides scope for intervention strategies which aim at a shift from a Th1 to a Th2 pattern. In experimental allergic encephalomyelitis (the animal model of multiple sclerosis), in non-obese diabetic mice (the animal model of diabetes mellitus), and in collagen-induced arthritis (an animal model of RA), a suppressive effect of Th2 clones (10), IL-4 (19), and the IL-4 gene (20) has been demonstrated. In this context, oral tolerance would work via local production ("bystander suppression") of Th2-type cytokines, which are believed to down-regulate the damaging effect of arthritogenic Th1 cytokines.

Recently, the first clinical trial of oral type II collagen in the treatment of RA demonstrated a trend toward improvement in the type II collagen–treated group compared with the placebo group (21). The design of the present study had three important differences from this earlier study: 1) only patients with early RA (disease duration ≤3 years) were included; 2) 2 different doses of type II collagen (1 mg and 10 mg) were compared with placebo; and 3) instead of chicken type II collagen we used bovine type II collagen, which has a higher homology to human type II collagen. In this study we found a slightly higher response rate among type II collagen–treated patients compared with those who received placebo, especially in the 10 mg group, although this difference was not significant.

PATIENTS AND METHODS

Patient recruitment and characteristics. Patients were recruited at 5 rheumatology clinics in Berlin. Inclusion criteria were as follows: 1) a diagnosis of RA according to the 1987 criteria of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) (22); 2) disease duration ≤3 years; 3) no treatment with disease-modifying antirheumatic drugs (DMARDs) in the previous 2 weeks; 4) clinically active RA with at least 4 swollen and tender joints; 5) treatment with a second-line drug according to need assessed by the physician; 6) prednisolone dosage ≤7.5 mg/day during the trial and for the 14 days before the trial, and no intraarticular injections of corticosteroids during the trial; 7) disease onset between the ages of 16 and 65; 8) patient's written consent.

Patients were excluded from the study if they had myocardial insufficiency, renal insufficiency (serum creatinine >2.0 mg/dl), disturbance of liver function (alkaline phosphatase >300 units/liter, serum glutamic oxaloacetic transaminase [SGOT] >50 units/liter, or bilirubin >1.5 mg/dl), malignancy, or a considerably reduced general state of health as determined by the physician.

Design and duration of the study. The study was a controlled, randomized, double-blind, phase II trial that included 3 groups of patients: 2 different dosage regimens of oral bovine type II collagen (1 mg and 10 mg) were tested against placebo. The planned study size was 30 patients per study arm. Study duration was 12 weeks. The study protocol was approved by the ethical committee of the Klinikum Benjamin Franklin of the Freie Universität Berlin.

Concomitant medication. All patients were treated with nonsteroidal antiinflammatory drugs (NSAIDs) throughout the trial. Any change in dosage or preparation was recorded.

Clinical and laboratory assessments. All patients were examined at 0, 4, 8, and 12 weeks after the start of treatment. The following clinical disease variables were assessed (23,24): number of painful joints (28-joint count), number of swollen joints (28-joint count), patient's global assessment of pain on a 10-point numerical rating scale, the Funktionsfragebogen Hannover (FFbH) questionnaire for measuring functional disability (25), patient's global assessment of disease activity on a 10-point rating scale, physician's global assessment of change in disease activity at the end of the treatment (on a 5-point rating scale), and erythrocyte sedimentation rate (ESR). We decided to use the FFbH questionnaire for measuring physical function because it is the best-validated instrument for RA patients living in Germany. The applied version consists of 12 questions concerning activities of daily living. Patients answer on a 3-point scale: 1 = yes, 2 = yes but with difficulty, 3 = no or only with help. Based on these responses, a score of 0 (no function) to 100% (unimpaired function) is generated. The Ritchie articular index (RAI) (26), duration of morning stiffness, grip strength (mean value of 3 measurements with a Martin vigorimeter), rheumatoid factor (RF), and C-reactive protein (CRP) levels were also documented. Radiographs obtained at study entry were reviewed retrospectively by one examiner.

RF was determined by quantitative nephelometric measurement of latex agglutination (N Latex RF; Behring AG, Marburg, Germany). A level >40 units was regarded as positive. CRP was determined by quantitative nephelometric measurement (N Latex CRP reagents; Behring AG). A value >6 mg/ml was considered positive. HLA class II typing was performed by sequence-specific primed polymerase chain reaction (PCR) (27) and consecutive sequencing-based typing after group-specific PCR amplification (28).

To monitor for possible side effects, the following variables were investigated at each visit: subjective condition, clinical status (measurement of weight and temperature, auscultation of heart and lung, abdominal palpation for tenderness and/or resistance, arterial blood pressure mea-

Table 1. Baseline characteristics of the 90 rheumatoid arthritis patients, by treatment group

Characteristic*	Placebo group (n = 30)	1-mg type II collagen group (n =30)	10-mg type II collagen group (n = 30)
Age, mean ± SD years	53 ± 13	52 ± 8	49 ± 11
No. female/no. male	25/5	20/10	22/8
Disease duration, mean ± SD months	10.3 ± 7.8	20.2 ± 12.0	12.3 ± 12.9
Functional status, no.†			
Class I	3	2	3
Class II	13	18	18
Class III	12	10	9
Class IV	1	0	0
Elevation of ESR, yes/no	15/15	22/8	13/17
Elevation of CRP, yes/no‡	16/14	20/10	13/17
RF positive/negative‡	14/16	17/13	16/14
RA-associated HLA subtype, yes/no‡	16/14	19/11	16/14
Prednisolone treatment, yes/no§	8/22	7/23	12/18
Previous DMARD treatment, no.			
None	28	20	22
Gold salts	–	2	–
Auranofin	–	1	–
Methotrexate	–	2	4
Hydroxychloroquine	–	–	1
Sulfasalazine	2	5	3

* ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; RA = rheumatoid arthritis; DMARD = disease-modifying antirheumatic drug.
† According to the revised criteria of the American College of Rheumatology (ref. 53).
‡ As defined in Patients and Methods.
§ Up to 7.5 mg.

surement, and examination of the skin), complete blood cell count including thrombocytes, levels of SGOT, serum glutamic pyruvic transaminase, alkaline phosphatase, gamma glutamyl transferase, and creatinine, and urinalysis.

Criteria for response. According to the definition proposed by the ACR (29), improvement in RA in clinical trials requires a ≥20% improvement in both tender joint count and swollen joint count and an additional ≥20% improvement in at least 3 of the following 5 parameters: patient global assessment of disease activity, patient pain assessment, patient self-assessed disability, physician global assessment of disease activity, and acute-phase reactant (ESR or CRP). For the FFbH, a change of ≥11% has been found to be significant by test–retest comparison (25), so this cutoff was used to designate a ≥20% improvement, and a change of ≥22% to designate a ≥40% improvement. The percentage change for the variables tender and swollen joints, ESR, and FFbH refers to the difference between the value at the end of study and the value at entry. Patient assessments of disease activity and of pain were measured on a 10-point scale. A difference of 1 point corresponded to 10%. A modification was made with regard to the physician's assessment of disease activity. At the end of the study the physician assessed the change in disease activity on a 5-point-scale (1 = much improved, 2 = improved, 3 = unchanged, 4 = deteriorated, 5 = much deteriorated). The value of 2 (improved) corresponded to an improvement of 20%, and the value 1 (much improved) to an improvement of 40%.

Preparation and handling of bovine type II collagen. Isolation of bovine type II collagen from the nasal septum of cows was carried out according to described methods (30,31). The isolated type II collagen was lyophilized and stored at −20°C. For the trial, type II collagen was dissolved in 0.5*M* acetic acid to final concentrations of 0.2 mg type II collagen/ml 0.5*M* acetic acid or 2 mg type II collagen/ml 0.5*M* acetic acid. Vials containing either 5 ml of 0.2 mg type II collagen/ml 0.5*M* acetic acid (1 mg type II collagen group), 5 ml of 2 mg type II collagen/ml 0.5*M* acetic acid (10 mg type

Table 2. Side effects, by treatment group

	Placebo group (n = 30)	1-mg type II collagen group (n = 30)	10-mg type II collagen group (n = 30)
Pruritus	2	2	3
Exanthema	2	0	3
Gastrointestinal symptoms	4	2	4
Headache	0	0	2

Table 3. Number (%) dropouts, by treatment group

	Placebo group (n = 30)	1-mg type II collagen group (n = 30)	10-mg type II collagen group (n = 30)
All dropouts	6 (20)	4 (13)	4 (13)
Dropouts due to disease worsening	5 (17)	4 (13)	3 (10)
Dropouts due to side effects	1 (3)	0 (0)	1 (3)

Table 4. Number (%) responders and nonresponders, by treatment group

Outcome	Placebo group (n = 30)	1-mg type II collagen group (n = 30)	10-mg type II collagen group (n = 30)
Improvement ≥20%*	4 (13)	6 (20)	7 (23)
Improvement ≥40%*†	0 (0)	1 (3)	3 (10)
Unchanged or worsening	26 (87)	24 (80)	23 (77)

* According to the American College of Rheumatology criteria (ref. 29).
† Includes those whose conditions improved by ≥20%.

II collagen group), or 5 ml 0.5*M* acetic acid (placebo group) were distributed once a month to the patients, who kept them in their home refrigerators at 6°C. Every morning patients swallowed this solution diluted in a glass of water (~100 ml), together with a multivitamin preparation to improve the taste.

Statistical analysis. All patients who were enrolled in the study were included in the evaluation. Before unblinding, the following specifications of the study protocol were determined: the response criteria proposed by the ACR (29) would be used for assessment of efficacy; each patient who fulfilled the criteria would be counted as a responder; and all other patients, including those who did not complete the trial, would be counted as nonresponders. The response rates were compared by Fisher's 1-tailed exact test (significance level 5%). If the response rate was comparable with that of methotrexate or gold, the test would have a power of at least 80% (29).

Additionally, mean changes between baseline and the end of the trial were described for the following efficacy parameters: tender joint count, swollen joint count, RAI, ESR, pain and disease activity assessment by the patients, functional capacity, morning stiffness, and grip strength. Analysis of covariance (ANCOVA) was used to compare these parameters, in order to detect any differences between groups. Individual differences (baseline value minus outcome) were adjusted for their starting point according to the model equation of ANCOVA, and these adjusted differences were then evaluated with two 1-tailed nonparametric tests. The Mann-Whitney test and the multivariate O'Brien test (32) were applied. In all tests, P values less than 0.05 were considered significant.

The statistical analysis included all 90 patients. An analysis of only those who completed the trial might have biased the results. We tested a simple method of replacing missing values (substituting the last valid value) and a more complex one (extrapolation by regression). Since the results were the same, the tables present only the first set of data. No replacement was made in the case of the FFbH because the FFbH was answered only twice, so a missing last value could not be estimated.

RESULTS

Patient characteristics at study entry. Ninety patients were enrolled in the study (30 in each group), of whom 67 (74.4%) were women and 23 (25.6%) were men. The mean age was 51 years (range 19–68). Seventy patients (78%) had not been treated previously with any DMARDs. Only 2 patients in the placebo group had received DMARD treatment before the beginning of the study, in comparison with 10 patients in the 1-mg type II collagen group and 8 patients in the 10-mg type II collagen group. Radiographs from the time of study entry, available for 66 patients, were examined retrospectively by one observer. Twenty-three patients exhibited erosions and 3 patients had joint space narrowing as radiologic signs of cartilage destruction. Table 1 summarizes the patients' characteristics at the start of the trial. The mean duration of disease, the number of patients with elevated ESR or CRP, the number of RF-positive patients, and the number of patients having an RA-associated HLA class II subtype (DRB1*0101, 0401, 0404, 0405, and 0408 [33]) were nonsignificantly higher in the 1-mg type II collagen group compared with the 10-mg type II collagen group and the placebo group. The groups were statistically similar in their demographic and clinical characteristics.

Side effects and withdrawals. Side effects were mild and were equally distributed among the groups (Table 2). Only 2 patients had to be withdrawn from the study because of a side effect (nausea in both cases), and 12 patients were withdrawn due to lack of efficacy (Table 3). Both the 1-mg and the 10-mg collagen treatment groups had fewer withdrawals due to disease worsening (4 patients and 3 patients, respectively) compared with the placebo group (5 patients).

Responders. Eighteen of the 90 patients had improvement according to the ACR criteria. One of the placebo group patients was not treated with prednisolone at study entry, but was treated with 7.5 mg prednisolone daily from week 4 until week 12 during the trial. This patient was not counted as a responder although the improvement criteria as described above were fulfilled.

There was no significant difference in response rates among the 3 groups, although more patients fulfilling the ACR criteria for 20% improvement were found in the 2 type II collagen groups than in the placebo group: 4 patients in the placebo group, 6 patients in the 1-mg type II collagen group, and 7 patients in the 10-mg type II collagen group responded (Table 4). Furthermore, 3 patients in the 10-mg type II collagen group and 1 patient in the 1-mg type II collagen group showed a very good response, either with an improvement of ≥40% by the ACR criteria

(patients 4, 6, and 12; Table 5) or with no swollen or tender joints at the end of treatment (patient 2), but no patient in the placebo group responded in this way. The responder patients are described in more detail in Table 5. No remission as defined by the ACR criteria for complete remission (34) occurred in any of the groups. In most cases the improvement started after 4 weeks, and usually showed a steady improvement over the 12-week treatment period (data not shown). In comparisons of the single variables, there were no significant differences between the groups (Table 6).

DISCUSSION

Statistically significant differences were not found in this study comparing treatment with 10 mg type II collagen/day, 1 mg type II collagen/day, and placebo, in terms of either the ACR criteria for improvement in RA (29) or the mean differences in single variables. However, we found that RA patients treated with type II collagen tended to show benefit. This was especially true with the higher dose of type II collagen (10 mg). In the 10-mg treatment group, 7 patients showed at least a 20% improvement, compared with 6 patients in the 1-mg group and 4 in the placebo group. The rate of withdrawals due to disease deterioration was greatest in the placebo group. It is especially worth mentioning that 4 patients in the type II collagen–treated group, but no patient in the placebo group, showed a very good response: 2 patients in the 10-mg collagen group and 1 in the 1-mg collagen group had improvement of at least 40%, and another patient in the 10-mg group had no tender or swollen joints at the end of the study. This is similar to the finding of 4 remissions in the type II collagen–treated group studied by Trentham et al (21).

Our results appear to be in accordance with those of the previous published study on treatment with oral type II collagen in RA (21), despite a different study design. We did not find a significant difference for any of the single variables, and the differences described in Trentham's study were also unimpressive. The salient feature of both studies seems to be the good response, with remission or near-remission, in a minority of patients. It does not seem to make much difference whether chicken or bovine type II collagen is used or whether early or late RA is treated, although no data on the duration of disease among the patients with remission were given in Trentham's report. Our findings indicate that the higher dosage of type II collagen, i.e., 10 mg/day, may be superior to the lower dosage. However, the differences between groups were too small to allow clear conclusions. It should be noted that the patients in the 1-mg type II collagen group had slightly more severe disease at study entry, as judged from the clinical data (Table 1). In most cases, there was a continuous improvement over the 12 weeks among the responders. This could be taken as evidence that a longer treatment period might be more favorable.

In terms of future studies, it is notable that we found no severe side effects among the collagen-treated patients, and that those that were found did not differ among the groups. Furthermore, the number of dropouts was smaller in the type II collagen groups than in the placebo group, making it unlikely that this treatment worsens the disease, although this is a theoretical possibility since absorption of immunogens in the gut is possible (35–37).

If only a minority of patients respond to treatment, it becomes difficult to demonstrate a significant effect. Our findings pose the question of why only a small number of patients respond, and whether these potential responders could be identified prior to treatment. No differences in RF positivity, HLA class II antigens, or other variables were detected between responders and nonresponders, so it is doubtful that these factors play a major role.

It may be that some patients handle type II collagen in the gut differently. A number of possibilities are worth considering in this context. The amount of oral antigen and the nature of the fragments generated seem to be crucial for the induction of oral tolerance. Digestion of the antigen in the gut is essential for the generation of oral tolerance (37,38), and interference with gastrointestinal proteolysis by neutralizing gastric pH might inhibit the induction of oral tolerance (35). The digestion of such a complex molecule as type II collagen might differ considerably between individuals, and it is not yet clear where and how type II collagen is digested in the gastrointestinal tract. This problem might be circumvented in the future if immunodominant type II collagen peptides can be identified and fed or inhaled (39), instead of administering the whole protein.

Furthermore, it has been suggested that induction of oral tolerance might be dependent on an intact mucosa, since a damaged mucosa allows the passage of immunogenic macromolecules (35,37). This could prevent oral tolerance in RA, since nearly all patients are treated with NSAIDs, which have a known mucosa-damaging effect (40,41). Another possibility is that a change of the bacterial gut flora, for example by drugs (42,43) or diet (44), could also influence the

Table 5. Clinical and laboratory parameters of efficacy and patient characteristics in responders*

Patient no./sex	Tender joints	Swollen joints	ESR	Pain	Disease activity	FFbH	Physician assessment	RF	HLA class II DRB1	Disease duration, months	Radiologic findings	Previous DMARD (washout phase)
10 mg type II collagen group												
Patient 1/F												
Baseline	15	12	48	3	3	–						
Study end	9	8	43	1	0	100.0	2	+	0401	36	Erosions in feet	MTX (25 days)
Difference	6.0	4.0	5.0	2.0	3.0	–						
Difference in %	40.0	33.3	10.4	20.0	30.0	–	20					
Patient 2/F												
Baseline	17	12	15	6	5	91.7						
Study end	0	0	10	2	2	95.8	2	–	†	32	No erosions	MTX (5 months)
Difference	17.0	12.0	5.0	4.0	3.0	4.2						
Difference in %	100.0	100.0	33.3	40.0	30.0	<20	20	–				
Patient 3/F												
Baseline	10	8	24	5	5	75.0						
Study end	8	5	27	2	2	83.3	2	–	0101	7	No erosions	None
Difference	2.0	3.0	−3.0‡	3.0	3.0	8.3						
Difference in %	20.0	37.5	−12.5‡	30.0	30.0	<20	20					
Patient 4/F												
Baseline	17	14	28	6	5	25.0						
Study end	1	2	17	1	2	66.7	1	+	0401	16	No erosions	None
Difference	16.0	12.0	11.0	5.0	3.0	41.7						
Difference in %	94.1	85.7	39.3	50.0	30.0	≥40	40					
Patient 5/F												
Baseline	16	10	10	4	4	75.0						
Study end	5	2	12	1	1	95.8	1	–	†	8	No erosions	None
Difference	11.0	8.0	−2.0‡	3.0	3.0	20.8						
Difference in %	68.8	80.0	−20.0‡	30.0	30.0	≥20	40					
Patient 6/F												
Baseline	20	20	8	6	8	66.7						
Study end	10	0	8	2	2	83.3	1	–	†	3	No erosions	None
Difference	10.0	20.0	0	4.0	6.0	16.7						
Difference in %	50.0	100.0	0	40.0	60.0	≥20	40					
Patient 7/F												
Baseline	15	10	6	1	4	100.0						
Study end	9	6	4	3	2	100.0	2	–	0101	4	No erosions	None
Difference	6.0	4.0	2.0	−2.0‡	2.0	0						
Difference in %	40.0	40.0	33.3	−20.0‡	20.0	<20	20					
1 mg type II collagen group												
Patient 8/F												
Baseline	14	16	83	8	8	50.0						
Study end	5	12	74	4	4	66.7	2	+	0101	11	Erosions in hands	None
Difference	9.0	4.0	9.0	4.0	4.0	16.7						
Difference in %	64.3	25.0	10.8	40.0	40.0	≥20	20					
Patient 9/M												
Baseline	12	10	41	6	7	87.5						
Study end	5	4	7	3	4	91.7	2	+	0401	11	No erosions	AUR (10.5 months)
Difference	7.0	6.0	34.0	3.0	3.0	4.2						
Difference in %	58.3	60.0	82.9	30.0	30.0	<20	20					
Patient 10/F												
Baseline	17	15	25	5	6	50.0						
Study end	0	8	26	2	2	70.8	2	–	0101	12	No erosions	None
Difference	17.0	7.0	−1.0‡	3.0	4.0	20.8						
Difference in %	100.0	46.7	−4.0‡	30.0	40.0	≥20	20					
Patient 11/M												
Baseline	3	9	22	5	7	79.2						
Study end	1	1	17	5	5	91.7	2	–	0401	6	No erosions	SSZ (18 days)
Difference	2.0	8.0	5.0	0	2.0	12.5						
Difference in %	66.7	88.9	22.7	0	20.0	≥20	20					

Table 5. *(Cont'd)*

Patient no./sex	Tender joints	Swollen joints	ESR	Pain	Disease activity	FFbH	Physician assessment	RF	HLA class II DRB1	Disease duration, months	Radiologic findings	Previous DMARD (washout phase)
Patient 12/F												
Baseline	28	16	30	6	4	87.5						
Study end	2	0	10	2	1	100.0	1	–	0101	7	No erosions	None
Difference	26.0	16.0	20.0	4.0	3.0	12.5						
Difference in %	92.9	100.0	66.7	40.0	30.0	≥20	40					
Patient 13/F												
Baseline	21	12	36	5	5	62.5						
Study end	6	2	18	3	2	79.2	1	–	0401	10	No erosions	None
Difference	15.0	10.0	18.0	2.0	3.0	16.7						
Difference in %	71.4	83.3	50.0	20.0	30.0	≥20	40					
Placebo group												
Patient 14/F												
Baseline	13	12	11	3	3	91.7						
Study end	0	6	7	0	0	100	2	–	0401	20	No x-ray available	None
Difference	13.0	6.0	4.0	3.0	3.0	8.3						
Difference in %	100.0	50.0	36.4	30.0	30.0	<20	20					
Patient 15/F												
Baseline	17	10	30	8	4	41.7						
Study end	6	4	23	6	4	50.0	2	+	0401	6	No x-ray available	None
Difference	11.0	6.0	7.0	2.0	0	8.3						
Difference in %	64.7	60.0	23.3	20.0	0	<20	20					
Patient 16/F												
Baseline	6	4	44	5	6	79.2						
Study end	3	3	36	2	3	91.7	2	+	0101, 0401	26	No x-ray available	SSZ (7 months)
Difference	3.0	1.0	8.0	3.0	3.0	12.5						
Difference in %	50.0	25.0	18.2	30.0	30.0	≥20	20					
Patient 17/F												
Baseline	8	12	22	5	5	87.5						
Study end	6	6	6	3	3	95.8	2	–	0401	10	No erosions	None
Difference	2.0	6.0	16.0	2.0	2.0	8.3						
Difference in %	25.0	50.0	72.7	20.0	20.0	<20	20					

* Tender and swollen joint values are from a 28-joint count. Pain and disease activity were rated on a 10-point scale. Physician assessment was done on a 5-point scale. ESR = erythrocyte sedimentation rate (mm/hour); FFbH = Funktionsfragebogen Hannover questionnaire (results expressed as percentage of full functional capacity; difference in % as defined in Patients and Methods); RF = rheumatoid factor; DMARD = disease-modifying antirheumatic drug; MTX = methotrexate; AUR = auranofin; SSZ = sulfasalazine.
† HLA subtype not associated with RA.
‡ Negative difference indicates deterioration.

effect of oral tolerance. It has been shown that orally administered lipopolysaccharide, a major component of bacterial cell walls, enhances the induction of oral tolerance (45), and the gut flora of untreated RA patients is significantly different from that of non-RA controls (46). Finally, treatment of animals with IFNγ abrogates oral tolerance (47). This could be taken as evidence that a strong Th1 response counteracts the assumed Th2 response induced by oral tolerance. Various influences can induce or increase a concomitant Th1 response, e.g., viral and bacterial infections or a hormonal imbalance, with glucocorticoids favoring a Th2 response and dehydroepiandrosterone sulfate and its derivative dehydroepiandrosterone favoring a Th1 response (48).

An additional factor as to why only a minority of patients responded could be our patient selection. We chose to treat only RA patients with early arthritis (duration ≤3 years). Since RA normally has a chronic course with eventual destruction of the joint, a treatment that is started early in the disease has a better chance of cure and/or of preventing irreversible joint damage. Furthermore, animal experiments indicate that disease is best prevented if antigen feeding starts before induction of disease or early in disease. However, the main disadvantage of concentrating on early

Table 6. Disease variables in the 2 type II collagen groups and the placebo group*

Variable, group	Mean ± SD value at entry	Difference from entry at week†			Adjusted difference‡
		4	8	12	
ACR core set variables					
Tender joints (28-joint count)					
Placebo	13.8 ± 6.1	2.2 ± 4.9	3.1 ± 5.2	2.7 ± 7.6	3.4 ± 6.7
1 mg collagen	15.7 ± 7.5	3.4 ± 5.7	5.8 ± 6.7	5.3 ± 7.2	5.1 ± 6.4
10 mg collagen	16.3 ± 5.9	2.3 ± 4.8	4.2 ± 7.3	3.5 ± 7.4	3.0 ± 7.1
Swollen joints (28-joint count)					
Placebo	11.3 ± 5.0	1.4 ± 4.0	1.6 ± 3.9	0.8 ± 4.7	1.0 ± 5.1
1 mg collagen	12.6 ± 5.0	1.7 ± 4.4	3.2 ± 5.1	3.3 ± 5.2	3.2 ± 4.9
10 mg collagen	12.5 ± 5.0	2.4 ± 4.7	2.1 ± 6.6	2.6 ± 7.5	2.5 ± 7.0
ESR (mm/hour)					
Placebo	31.9 ± 29.4	0.9 ± 15.7	2.5 ± 11.5	3.3 ± 12.6	3.5 ± 13.5
1 mg collagen	40.9 ± 31.2	7.6 ± 18.3	12.0 ± 24.2	10.0 ± 24.0	8.3 ± 20.1
10 mg collagen	26.5 ± 21.5	−4.2 ± 14.5	−7.8 ± 16.8	−2.5 ± 13.8	−1.2 ± 15.5
Patient assessment of disease activity (10-point rating scale)					
Placebo	5.2 ± 1.8	1.3 ± 2.3	1.1 ± 1.9	1.0 ± 2.0	0.9 ± 1.9
1 mg collagen	4.5 ± 2.4	0.5 ± 2.1	0.1 ± 2.7	0.1 ± 2.7	0.1 ± 2.3
10 mg collagen	4.8 ± 1.9	0.1 ± 1.9	−0.2 ± 2.6	0.0 ± 2.6	0.0 ± 2.5
Patient pain assessment (10-point rating scale)					
Placebo	5.4 ± 2.1	0.8 ± 2.4	1.0 ± 2.1	1.0 ± 1.9	0.6 ± 2.2
1 mg collagen	4.5 ± 2.1	−0.3 ± 2.8	0.0 ± 2.6	0.4 ± 2.0	0.0 ± 2.4
10 mg collagen	5.0 ± 1.9	−0.3 ± 2.6	−0.3 ± 2.6	−0.1 ± 1.5	−0.2 ± 2.5
Patient self-assessed disability (FFbH)§					
Placebo	71.8 ± 18.2			5.7 ± 12.6	
1 mg collagen	76.7 ± 16.9			5.0 ± 13.1	
10 mg collagen	76.0 ± 16.8			1.4 ± 14.8	
Physician assessment of change in disease activity (5-point scale)¶					
Additional variables					
Ritchie articular index					
Placebo	15.4 ± 6.8	2.8 ± 6.6	3.9 ± 9.3	2.6 ± 9.8	3.7 ± 9.9
1 mg collagen	18.6 ± 8.3	4.9 ± 7.4	6.7 ± 10.3	5.6 ± 9.6	5.3 ± 8.1
10 mg collagen	19.4 ± 10.1	3.4 ± 6.4	4.7 ± 11.0	5.2 ± 11.0	4.5 ± 10.1
Morning stiffness (minutes)					
Placebo	117.2 ± 62.3	49.3 ± 53.1	35.0 ± 106.2	48.5 ± 66.6	41.0 ± 57.0
1 mg collagen	82.2 ± 82.3	3.4 ± 160.2	24.3 ± 89.2	26.2 ± 87.3	31.6 ± 73.4
10 mg collagen	98.2 ± 68.6	30.2 ± 54.7	23.9 ± 88.2	49.0 ± 64.3	48.5 ± 49.5
Grip strength (kPa), right hand					
Placebo	36.7 ± 20.7	8.4 ± 16.0	11.9 ± 17.3	8.7 ± 17.6	6.6 ± 16.5
1 mg collagen	51.4 ± 25.6	2.0 ± 13.4	0.5 ± 17.8	0.7 ± 17.9	2.1 ± 17.1
10 mg collagen	50.3 ± 26.5	−4.6 ± 11.0	−0.9 ± 17.4	1.9 ± 20.7	3.0 ± 19.9
Grip strength (kPa), left hand					
Placebo	23.7 ± 16.4	21.8 ± 16.1	22.8 ± 18.0	20.3 ± 16.3	20.1 ± 16.3
1 mg collagen	37.2 ± 22.1	16.8 ± 12.6	16.2 ± 17.3	15.0 ± 17.8	15.2 ± 17.7
10 mg collagen	33.3 ± 19.3	13.8 ± 14.3	16.0 ± 16.0	17.1 ± 18.2	17.2 ± 18.3

* All patients who enrolled (including dropouts) were included in the analysis; n = 30 in each group. ACR = American College of Rheumatology; see Table 5 for other definitions.
† Negative difference indicates deterioration.
‡ Baseline minus week 12, as defined in Patients and Methods.
§ Applied only at study entry and end. Results expressed as percentage of full functional capacity; dropouts not included.
¶ At study end. In the placebo group, 1 patient's condition was judged to be much improved, 17 improved, 5 unchanged, 5 deteriorated, and 2 much deteriorated. In the 1 mg collagen group, 3 patients' conditions were judged to be much improved, 11 improved, 9 unchanged, 4 deteriorated, and 3 much deteriorated. In the 10 mg collagen group, 3 patients' conditions were judged to be much improved, 12 improved, 7 unchanged, 5 deteriorated, and 3 much deteriorated.

RA in a study like this lies in the possibility that non-RA patients may be inadvertently included. Although all patients in the study met at least 4 of the ACR criteria for RA, in the absence of erosions patients with other, RA-like, diseases are likely to be included, despite the reported 85% specificity of the criteria in early RA (22). Diseases that can resemble early RA, such as primary Sjögren's syndrome, do not

normally lead to erosions of cartilage/bone. However, the concept of oral tolerance in a disease like RA relies heavily on the effect of bystander suppression induced by type II collagen in the joint.

Normally, type II collagen is sequestrated at a privileged site, where it is not recognized by the immune system. Only in a cartilage-destroying disease like RA, with formation of new blood vessels and invasion of T cells and macrophages, can it be assumed that T cells migrating from the gut recognize and are stimulated by collagen in the joint. This would mean that a condition that is confined to inflammation in the synovium, without cartilage damage, would not benefit from treatment with oral type II collagen. Individuals with such conditions would probably be included in any group of patients believed to have early RA. In our study, erosions were not a feature that differentiated responders from nonresponders, but radiologic criteria may be less informative early in disease.

An important means of identifying which RA patients are likely to respond to oral/nasal treatment with type II collagen could be their own level of T cell reactivity to the protein prior to the start of treatment. For this purpose it would help to identify one or more immunodominant peptides, because such peptides have proved useful in assessing T cell reactivity in other diseases (49,50). By way of comparison, mice in which arthritis is induced by foreign type II collagen are highly variable in the extent to which they develop reactivity to their own collagen (51). It would also be helpful if the successful induction of oral tolerance could be measured in patients or controls by the release of cytokines of peripheral blood lymphocytes after in vitro stimulation with type II collagen. Forty-eight to 52 hours after stimulation with myelin basic protein, TGFβ-secreting regulatory cells can be detected in Peyer's patches (52).

In conclusion, we found a tendency toward a higher response rate in the type II collagen–treated patients with RA, especially in the 10-mg treatment group, which is encouraging, and, in our opinion, justifies further investigations of oral tolerance in the treatment of this disease. Oral tolerance is an extremely interesting approach toward immunosuppression because it combines nontoxicity with antigen selectivity. Above all, we need to know how to identify which RA patients are likely to respond to oral tolerance therapy.

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REFERENCES

1. Sieper J, Mitchison AN: Therapy with oral collagen type II as new possibility for selective immunosuppression in the therapy of rheumatoid arthritis. Z Rheumatol 53:53–58, 1994
2. Weiner HL, Friedman A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, Sayegh M, Nussenblatt RB, Trentham DE, Hafler DA: Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu Rev Immunul 12:809–837, 1994
3. Nagler-Anderson C, Bober 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 83:7443–7446, 1986
4. Zhang JZ, Lee CSY, Lider O, Weiner HL: Suppression of adjuvans arthritis in Lewis rats by oral administration of type II collagen. J Immunol 145:2489–2493, 1990
5. Poole AR: Cartilage in health and disease. In, Arthritis and Allied Conditions. Twelfth edition. Edited by DJ McCarty, WJ Koopman. Philadelphia, Lea & Febiger, 1993
6. Prockop DJ, Williams CJ, Vandenberg P: Collagen in normal and diseased connective tissue. In, Arthritis and Allied Conditions. Twelfth edition. Edited by DJ McCarty, WJ Koopman. Philadelphia, Lea & Febiger, 1993
7. Desai BV, Dixit S, Pope RM: Limited proliferative response to type II collagen in rheumatoid arthritis. J Rheumatol 16:1310–1314, 1989
8. Terato K, Shimozuru Y, Katayama K, Takemitsu Y, Yamashita I, Miyatsu M, Fujii K, Sagara M, Kobayashi S, Goto M, Nishioka K, Miyasaka N, Nagai Y: Specificity of antibodies to type II collagen in rheumatoid arthritis. Arthritis Rheum 33: 1493–1500, 1990
9. Miller A, Lider O, Weiner HL: Antigen-driven bystander suppression after oral administration of antigens. J Exp Med 174:791–798, 1991
10. Chen Y, Kuchroo VK, Inobe J-I, Hafler DA, Weiner HL: Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 265:1237–1240, 1994
11. Panayi GS, Lanchbury JS, Kingsley GH: The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis (editorial). Arthritis Rheum 35:729–735, 1992
12. Firestein GS, Zvaifler NJ: The role of T cells in rheumatoid arthritis. Ann Rheum Dis 52:765, 1993
13. Simon AK, Seipelt E, Sieper J: Divergent T-cell cytokine patterns in inflammatory arthritis. Proc Natl Acad Sci U S A 91:8562–8566, 1994
14. Lindblad S, Ulfgren A-K, Klareskog L, Andersson U: Detection of cytokine-producing cells in the synovial membrane in rheumatoid arthritis (abstract). Arthritis Rheum 37 (suppl 9): S193, 1994
15. Steiner G, Akrad N, Kunaver M, Gal A, Wessely M, Zenz P, Smolen JS: Production of cytokines by T cells in RA synovial membranes. Clin Rheumatol 14:236, 1995
16. Khoury SJ, Hancock WW, Weiner HL: Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation to inflammatory cytokine and differential upregulation of

transforming growth factor beta, interleukin 4, and prostaglandin expression in the brain. J Exp Med 176:1355–1364, 1992
17. Shehadeh NN, La Rosa F, Lafferty KJ: Altered cytokine activity in adjuvant inhihibiton of autoimmune diabetes. J Autoimmun 6:291–300, 1993
18. Liblau RS, Singer SM, McDevitt HO: Th1 and Th2 CD4+ T cells in the pathogenesis of organ specific autoimmune diseases. Immunol Today 16:34–38, 1995
19. Rapoport MJ, Jaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, Cyopick P, Danska JS, Delovich TL: Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. J Exp Med 178:97–99, 1993
20. Bessis N, Boissier M-C, Caput D, Fradelizi D, Fournier C: IL-4 or IL-13 transfected xenogenic fibroblasts in the treatment of collagen induced arthritis in mice. Clin Rheumatol 14:261, 1995
21. Trentham DE, Dynesius-Trentham RA, Orav EJ, Combitchi D, Lorenzo C, Sewell KL, Hafler DA, Weiner HL: Effects of oral administration of type II collagen on rheumatoid arthritis. Science 261:1727–1730, 1993
22. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324, 1988
23. Felson DT, Anderson JJ, Boers M, Bombardier C, Chernoff M, Fried B, Furst D, Goldsmith C, Kieszak S, Lightfoot R, Paulus H, Tugwell P, Weinblatt M, Widmark R, Williams HJ, Wolfe F: The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. Arthritis Rheum 36:729–740, 1993
24. Prevoo MLL, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LBA, van Riel PLC: Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38:44–48, 1995
25. Raspe H-H, Kindel P, Vesterling K, Kohlmann T: Die Entwicklung der Funktionskapazität und der Schmerzintensität von 81 cP-Patienten unter einer Behandlung mit Azulfidine RA oder Aurothioglucose. Z Rheumatol 46:71–75, 1987
26. Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakos TG, Grieveson P, Buchanan WW: Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 37:393–406, 1968
27. Olerup O; Zetterquist H: HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 39:225–35, 1992
28. Blasczyk R, van Lessen A, Schwella N, Huhn D, Salama A: A novel HLA DR 13 allele (DRB1*1314) identified by single-strand conformation polymorphism analysis and confirmed by direct sequencing. Hum Immunol 43:303–312, 1995
29. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, Katz LM, Lightfoot R Jr, Paulus H, Strand V, Tugwell P, Weinblatt M, Williams HJ, Wolfe F, Kieszak S: American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 38:727–735, 1995
30. Ricard-Blum S, Tiollier J, Garrone R, Herbage D: Further biochemical and physiochemical characterization of minor disulfide-bonded (type IX) collagen, extracted from foetal calf cartilage. J Cell Biochem 27:147–158, 1985
31. Boissier M-C, Chiocchia G, Ronziere M-C, Herbage D, Fournier C: Arthritogenicity of minor cartilage collagens (types IX and XI) in mice. Arthritis Rheum 33:1–8, 1990
32. O'Brien PC: Procedures for comparing samples with multiple endpoints. Biometrics 40:1079–1087, 1984
33. Ollier W, Thomson W: Population genetics of rheumatoid arthritis. Rheum Dis Clin North Am 18:741–759, 1992
34. Pinals RS, Masi AT, Larsen RA, and the Subcommittee for Criteria of Remission in Rheumatoid Arthritis of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee: Preliminary criteria for clinical remission in rheumatoid arthritis. Arthritis Rheum 24:1308–1315, 1981
35. Bloch K, Bloch D, Stearns M, Walker WA: Intestinal uptake of macromolecules. VI. Uptake of protein antigen in vivo in normal rats and in rats infected with Nippostrongylus brasiliensis or subjected to mild systemic anaphylaxis. Gastroenterology 77:1039–1044, 1977
36. Curtis CH, Gall DG: Macromolecular transport by rat gastric mucosa. Am J Physiol 257:G1033–G1040, 1992
37. Hanson D, Roy M, Green GM, Miller S: Inhibition of orally-induced immune tolerance im mice by prefeeding an endopeptidase inhibitor. Reg Immunol 5:76–74, 1993
38. Michael JG: The role of digestive enzymes in orally induced immune tolerance. Immunol Invest 18:1049–1054, 1989
39. Metzler B, Wraith DC: Inhibiton of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalogenic peptide: influence of MHC binding affinity. Int Immunol 5:1159–1165, 1993
40. Bjarnason I, Williams P, So A, Zanelli GD, Levi A J, Gumpel JM, Peters TJ, Ansell B: Intestinal permeability and inflammation in rheumatoid arthritis: effects of non-steroidal anti-inflammatory drugs. Lancet 2:1171–1173, 1984
41. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ: Nonsteroidal antiinflammatory drug-induced intestinal inflammation in humans. Gastroenterology 93:480–489, 1987
42. Bradley SM, King B, Troughton PR, Gooi HC, Bird HA, Wright V: Clostridium perfringens and antibody responses to its antigen in arthritis patients on and off non-steroidal anti-inflammatory drugs. Br J Rheumatol 32:940–941, 1993
43. Kanerud L, Scheynius A, Nord CE, Haftström I: Effect of sulphasalazine on gastrointestinal microflora and on mucosal heat shock protein expression in patients with rheumatoid arthritis. Br J Rheumatol 33:1039–1048, 1994
44. Peltonen R, Kjeldsen-Kragh J, Haugen M, Tuominen J, Toivanen P, Forre O, Eerola E: Changes of faecal flora in rheumatoid arthritis during fasting and one-year vegetarian diet. Br J Rheumatol 33:638–643, 1994
45. Khoury SJ, Lider O, Al-Sabbagh A, Weiner HL: Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. III. Synergistic effect of lipopolysaccharide. Cell Immunol 131:302–310, 1990
46. Eerola E, Möttönen T, Hannonen P, Luukkainen R, Kantola I, Vuori K, Tuominen J, Toivanen P: Intestinal flora in early rheumatoid arthritis. Br J Rheumatol 33:1030–1038, 1994
47. Zhang Z, Michael JG: Orally inducible immune responsiveness is abrogated by IFNγ treatment. J Immunol 144:4163–4165, 1990
48. Clerici M, Shearer GM: The Th1-Th2 hypothesis of HIV infection: new insights. Immunol Today 15:575–581, 1994
49. Nishimura M, Kermode AG, Clerici M, Shearer GM, Berzofsky JA, Uchiyama T, Wiktor SZ, Pate E, Maloney B, Manns A, Blattner W, Jacobson S: Demonstration of human T lymphotropic virus type I (HTLV-1)-specific T cell responses from seronegative and polymerase chain reaction negative persons to HTLV-1. J Infect Dis 170:334–338, 1994
50. Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, McAdam S, Whitby D, Sabally S, Gallimore A, Corrah T,

Tariguchi M, Schultz T, McMichael A, Whittle H: HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nature Med 1:59–64, 1995

51. Dietrich A, Mitchison NA, Raynavolgyi E, Schneider SC: Primed lymphocytes are boosted by type II collagen of their hosts after adoptive transfer. J Autoimmun 7:601–609, 1994
52. Santos LMB, Al-Sabbagh A, Londono A, Weiner HL: Oral tolerance to myelin basic protein induces regulatory TGF-β secreting cells in Peyer's patches of SJL mice. Cell Immunol 157:439–447, 1994
53. Hochberg MC, Chang RW, Dwosh I, Lindsey S, Pincus T, Wolfe F: The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum 35:498–502, 1992