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CAN COLLAGEN TYPE II SUSTAIN A METHOTREXATE-INDUCED THERAPEUTIC EFFECT IN PATIENTS WITH LONG-STANDING RHEUMATOID ARTHRITIS? A DOUBLE-BLIND, RANDOMIZED TRIAL

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SUMMARY

Objective. Based on the results of two recently published, randomized, double-blind and placebo-controlled studies, a possible improvement in rheumatoid arthritis disease activity after oral tolerization with triple helical collagen type II has been suggested. The goal of this study was to go one step further and ask the question whether collagen type II can sustain the therapeutic effect induced by methotrexate, the most widely accepted disease-modifying anti-rheumatic drug in patients with long-standing rheumatoid arthritis.

Methods. Ninety-two patients with rheumatoid arthritis on stable therapy with methotrexate were enrolled in a 3 month double-blind, randomized and comparative study to examine the efficacy of oral triple helical collagen type II as compared to continuing methotrexate. The dose of methotrexate (or the respective placebo drug) and of concomitant corticosteroids was not changed and intra-articular corticosteroids were not allowed during the 3 months. The primary study endpoint was disease activity as measured by physician and patients.

Results. While patients under ongoing therapy with methotrexate had, as expected, no change in disease activity, almost all parameters of disease activity and outcome in patients under a daily oral dose of 0.5 mg triple helical collagen type II worsened significantly (highly significant difference in swollen joints, between the two groups, $P < 0.0001$). No significant differences in side-effects between the two groups during the study period could be demonstrated.

Conclusions. Substitution of methotrexate with daily 0.5 mg of triple helical collagen type II in patients with rheumatoid arthritis leads to a significant increase in disease activity, suggesting that oral collagen type II at the given dose is not capable of sustaining the methotrexate-induced anti-inflammatory effect in patients with long-standing rheumatoid arthritis.

KEY WORDS: Clinical trial, Comparative study, Oral immune tolerance, Collagen type II, Methotrexate, Rheumatoid arthritis.

ONE of the primary goals in developing effective therapy for autoimmune diseases is to suppress autoreactive immune processes specifically without affecting the remainder of the immune system. Short-term studies and meta-analyses have repeatedly proven the efficacy of disease-modifying anti-rheumatic drugs (DMARDs), but many patients take them for <5 yr because of either lack of efficacy or toxic effects [1]. Regarding methotrexate (MTX), one of the most widely used DMARDs in rheumatoid arthritis (RA), only 30% of patients in a large cohort were taking it for >10 yr, and 50% discontinued it due to toxicity [2].

Oral tolerance therapy has long been recognized as being able to induce peripheral immune tolerance to specific antigens [3] and has been examined in animal studies [4]. Oral administration of pepsin-digested, triple helical collagen type II (COL II) has proved highly effective in various animal models of human autoimmune diseases, including collagen-induced arthritis [5, 6] and adjuvant arthritis [7]. Because of some similarities of these animal models to RA, and considering the abundance of COL II in articular cartilage [8], COL II has been chosen as an oral toleragen in clinical trials on patients with RA. There is no convincing evidence that COL II itself is the relevant mediator of disease activity in RA, but antigen-specific bystander suppression has been discussed 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 [7, 9].

Based on these rationales, Trentham *et al.* [10] performed a first placebo-controlled double-blind study with COL II from normal chickens in 60 patients with active RA in 1993. A significant improvement in painful and swollen joints could be shown in the patient group receiving collagen. In a second, placebo-controlled study in patients with early RA conducted by Sieper *et al.* [11], bovine COL II was given in higher doses (1 and 10 mg/day, respectively). Only a slightly higher (but non-significant) response rate among COL II-treated patients compared to placebo could be demonstrated.

The rationale of the present study was to apply several parameters used in the first controlled study by Trentham *et al.* and ask the question whether oral COL II can sustain an MTX-induced stable anti-inflammatory effect in patients with long-standing RA, who are dependent on this therapy. Here we report the results of a randomized, comparative double-blind study comparing COL II with MTX in 92 patients

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with active RA under a hitherto stable treatment with MTX.

PATIENTS AND METHODS

Study population

Patients were recruited by general practitioners (20%), rheumatologists with a private practice (55%) and at our out-patient clinic at the department of rheumatology (25%). Patients with a diagnosis of RA according to the 1987 criteria of the American College of Rheumatology (ACR) [12] who were on an ongoing and unchanged treatment for at least 8 weeks with MTX as the only DMARD, presenting an RA functional status of class I–III according to the ACR criteria [13], and who were on an unchanged dose of glucocorticosteroids of $\leqslant$12.5 mg/day and of non-steroidal anti-inflammatory drugs (NSAIDs) within the last 2 weeks before starting the study, were eligible for participation and included after written informed consent by the patient.

Patients were excluded from the study if $<$18 yr of age and in the case of intra-articular glucocorticosteroid use within 3 weeks before entering the study.

Study design and treatment

Patients were randomly assigned in a double-blind fashion to receive either COL II or MTX for 3 months. The minimal sample size n was 40 patients per treatment group based on a power of 90% (β of 0.1; α of 0.05) to detect a 30% difference in swollen joints between the MTX and the COL II treatment group [14]. The study protocol was approved by the ethical committee of the University Hospital Zürich.

Treatment regimens were as follows. Group 1: 0.5 mg COL II and MTX placebo (according to the patient's previous dose and route of application); group 2: identical dose and application of verum MTX as used before study entry and placebo collagen.

COL II. Collagen type II was isolated (PB) by limited pepsin digestion of sternal cartilage from commercial 40-day-old male broiler chickens. The protein was purified as published earlier [15]. The purity of the protein was judged by gel electrophoresis and exclusively produced bands corresponding to polypeptides of COL II were the accepted standard. After purification, the protein was dissolved in 0.1 M acetic acid and solutions containing 0.5 mg of COL II were mixed with 120 ml of heat-inactivated orange juice, containing 0.15% sodium benzoate (stabilizer). The volume was filled in tetra packages and stored until use at 4–8°C. During the study, all patients stored their collagen at the same temperature throughout the whole study period.

Chicken COL II was purified identically as reported by the group of Trentham [10]. Even if bovine COL II has a higher homology to human COL II, we decided to use the chicken species for two reasons. First, the study by Trentham also used chicken COL II and, in the light of bovine spongiform encephalopathy, the use of bovine COL II in humans was considered unpredictable and therefore not safe.

Collagen type II placebo. This consisted of 120 ml of heat-inactivated orange juice, containing 0.15% sodium benzoate (stabilizer), filled in tetra packages and stored until use at 4–8°C.

MTX verum and placebo tablets à 2.5 mg. These were supplied by Wyeth-Lederle Pharmaceutical Company, Zug, Switzerland. Verum and placebo MTX for parenteral use was delivered by Bristol Myers Pharmaceutical Company, Baar, Switzerland.

Concomitant medication

Doses of NSAIDs and glucocorticosteroids were kept constant for the entire trial period. A change in glucocorticosteroid dose as well as intra-articular or i.m. injections of glucocorticosteroids during the study were considered a protocol violation leading to termination of the study. In the case of an exacerbation of disease activity, an augmentation of NSAIDs to maximal recommended doses and additional paracetamol up to 4 g/day was allowed as escape medication.

Data collection and measures

For all patients, three study visits were determined (day 0, 30 days and 90 days after enrolment) and examination performed by the same clinical investigator, who was unaware of the treatment group assignments.

Patient characteristics (Table I). Sociodemographic data were recorded at day 0, clinical and laboratory examinations at day 0, 30 and 90. Laboratory measurements included Westergren erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF). HLA class II typing was performed by sequence-specific primed polymerase chain reaction (PCR) [16] and consecutive sequencing-based typing after group-specific PCR amplification [17]. RA-associated HLA class II subtypes were defined according to the published reports of Ollier and Thomson [18] and Weyand *et al*. [19] (Table I).

New radiographs of both hands and feet were reviewed in a blinded manner for the presence or absence of RA-related erosions.

The following clinical disease variables were assessed at each study visit.

Primary endpoints. Disease activity as assessed by the physician (number of tender and swollen joints out of 28 joints). In addition, disease activity was calculated using the number of tender and swollen joints, and ESR by applying the algorithm of the disease activity score [20–24] as assessed by the physician (DAS) and the patient (RA disease activity index, RADAI) [25–27]. In both scores (DAS and RADAI), the value of 10 is the highest possible disease activity.

Seven secondary endpoints. (1) Other clinical parameters of disease activity including morning stiffness [24, 25], grip strength on both sides with use of a Martin vigorimeter, and muscle strength [28] as assessed by the physician. (2) Symptom severity by assessment of pain on a numerical rating scale from 0 to 10 (NRS) [29] by the patients. (3) Physical functional status as assessed by the physician (ACR) [13]

TABLE I
Baseline characteristics of 92 patients with rheumatoid arthritis, randomly assigned to receive methotrexate or collagen type II*

Characteristics	Methotrexate ($n = 46$)	COL II ($n = 46$)
Age (yr) mean	50.3 ± 13.0	53.4 ± 12.0
Sex (F/M)	41/5	35/11
Disease duration (months)	119 ± 97	143 ± 117
Functional status (no. of patients)†		
Class I	5	11
Class II	13	15
Class III	28	20
Class IV	0	0
DAS at entry‡	4.33 ± 1.29	4.25 ± 1.48
No. of patients in subgroups of DAS		
$\leqslant 2.4$	3	5
>2.4–$\leqslant 3.7$	14	9
>3.7	29	32
ESR (mm/1 h)	23 ± 20	21 ± 17
Rheumatoid factor (% positive)	69.6	69.6
RA-associated HLA-DR subtype (% positive)§	63.4	68.4
Radiological erosions (% positive)	98	100
Weekly dosage of MTX (mg)	13.1 ± 5.9	11.3 ± 5.1
Mean duration of MTX (months)	25.1 ± 25.3	23.2 ± 19.2
Steroid treatment (% yes)	43.5	45.7
Mean dosage of steroids/day (mg)	6.9 ± 8.5	6.1 ± 8.1
Number of previous DMARDs	5	5
NSAIDs at study entry (% yes)	80	79.6

* ± values are means ± S.D. There is no significant difference ($P \leqslant 0.05$) between the two treatment groups.
†ACR functional status of rheumatoid arthritis [13].
‡DAS, validated Disease Activity Index, using a maximum joint count of 28 (tender and swollen) in addition to ESR (see Patients and methods).
§HLA-DRB1*0101, 0401, 0404, 0405, 0408, 1402 (see Patients and methods).

and the patient [Health Assessment Questionnaire (HAQ), a score of 3 indicating greatest disability] [29–31]. (4) Health-related quality of life, as assessed by the patient (SF-36 mental and physical component score questionnaire, a score of 100 being the best possible value) [32–34]. (5) Assessment of general health by the physician and the patient on a numerical rating scale 0–10. (6) Overall assessment of the efficacy and tolerability of the drug by the patient and the physician on a five-point rating scale. (7) Increase in dose of NSAIDs as escape medication.

Case conclusion (successful termination or discontinuation of study). Case conclusion of each patient was classified and recorded either as regular termination of the study or premature discontinuation with subsequent registration of exact study duration and specific reason for drop-out. Each patient who dropped out had a final clinical and laboratory examination and a set of questionnaires (HAQ, RADAI, SF-36) to answer within 2 days after drop-out.

Monitoring for adverse events. To monitor for safety and possible adverse events, the following variables were investigated at each visit in addition to questioning about the presence, frequency, duration and intensity of adverse events: clinical status and complete blood cell count, renal and hepatic function tests, and urine analysis.

Statistical analysis

Differences among the two treatment groups at entry and differences in changes in variables of disease activity, symptom severity and short-term disease outcomes after 30 and 90 days were analysed using the χ^2 test for binary data and unpaired *t*-test for continuous data.

All patients who had at least two (regular or premature) study visits (first visit at day 0, second between day 0 and day 30) were included in the analysis of variables at day 30. All patients who had a third study visit between day 30 and day 90 (all patients including regular termination and drop-out) were considered for the analysis of variables at day 90 (intention-to-treat analysis). Comparisons between treatment groups were calculated using the Mann–Whitney *U*-test. A Kaplan–Meier curve was computed for all patients, assuming that patients who did not complete the entire study successfully had a treatment failure. The log rank test was used to compare the two groups. Statistical analysis was performed using Statview 4.5 (Abacus Concepts, Inc., Berkeley, CA, USA) and S-PLUS Version 3.3 (Stat Sci, a division of Math Soft, Inc., Seattle, WA, USA).

RESULTS

Baseline characteristics

Ninety-two patients on an established therapy with MTX were randomly assigned to receive daily 0.5 mg COL II (46 patients) or an unchanged dose of MTX (46 patients). There were no significant differences in baseline characteristics among the groups at study entry (Table I).

Treatment-related disease activity and short-term outcome measures

Table II shows the mean values and S.D. of disease activity (five variables) and of disease outcome (three variables) as assessed by the physician and by patients at baseline and after 1 and 3 months of therapy. After 3 months of therapy, there were significant differences in changes in four of five variables with respect to disease activity and in two of three variables with respect to disease outcome between the patient group receiving COL II and the MTX group.

Most impressive, after 3 months, the mean swollen joint count of the COL II group was 9.02 compared to 5.91 in the group receiving MTX, denoting a significant absolute difference in 3.68 swollen joints between the two groups with respect to their baseline values (15% improvement in the MTX group *vs* 41.2% deterioration in the COL II group). Lower, but still significant differences in mean changes between these two treatment groups could already be seen after 1 month. Regarding the ESR, the absolute difference in the mean changes between the two groups from their baseline values was 14 mm (5.5% decrease in the MTX group *vs* 60.4% increase in the COL II group). After

TABLE IIa
Baseline values and absolute changes in measures of disease activity and disease outcomes in 92 patients with rheumatoid arthritis according to the treatment group*

Variables and study visits†	Methotrexate	COL II	P‡ Methotrexate *vs* COL II
No. of patients			
Baseline visit	46	46	
Second visit (after 1 month)	45	44	
Third visit (after 3 months)	43	32	
Swollen joints (0–28)			
Baseline visit	6.96 ± 6.16	6.39 ± 5.43	0.88
Change at second visit	0.04 ± 2.75	1.71 ± 3.20	0.007
Change at third visit	−1.05 ± 3.07	2.63 ± 2.64	<0.0001
Tender joints (0–28)			
Baseline visit	6.26 ± 5.49	6.91 ± 6.98	0.95
Change at second visit	0.47 ± 3.02	2.11 ± 5.53	0.13
Change at third visit	−0.16 ± 3.65	1.00 ± 6.03	0.15
Erythrocyte sedimentation rate (mm/1 h)			
Baseline visit	23.4 ± 20.2	21.1 ± 16.9	0.57
Change at second visit	−0.2 ± 8.5	10.5 ± 15.7	0.0003
Change at third visit	−1.3 ± 14.1	12.7 ± 17.0	<0.0001
Disease activity score (DAS) (0–10)			
Baseline visit	4.33 ± 1.29	4.25 ± 1.48	0.77
Change at second visit	0.15 ± 0.53.	0.58 ± 0.84	0.004
Change at third visit	−0.19 ± 0.70	0.70 ± 1.14	0.0003
RA disease activity index (RADAI) (0–10)			
Baseline visit	2.65 ± 1.56	3.20 ± 2.40	0.21
Change at second visit	0.02 ± 1.82	1.03 ± 2.11	0.022
Change at third visit	−0.28 ± 1.67	1.00 ± 2.57	0.024
Physical functional status (HAQ) (0–3)			
Baseline visit	0.98 ± 0.55	0.92 ± 0.77	0.47
Change at second visit	−0.04 ± 0.37	0.18 ± 0.50	0.02
Change at third visit	−0.11 ± 0.44	0.25 ± 0.58	0.0007
Health-related quality of life (SF-36) (0–100)			
Physical component score			
Baseline visit	36.2 ± 10.6	36.3 ± 12.9	0.95
Change at second visit	0.98 ± 8.21	−2.25 ± 6.18	0.048
Change at third visit	2.17 ± 9.48	−5.20 ± 8.89	0.002
Health-related quality of life (SF-36) (0–100)			
Mental component score			
Baseline visit	51.3 ± 11.5	49.4 ± 11.3	0.42
Change at second visit	1.15 ± 9.07	−0.17 ± 10.32	0.59
Change at third visit	0.55 ± 8.19	1.22 ± 10.58	0.46

*± values are means ± S.D.

†Joint scores and other assessment variables are described in Patients and methods. Baseline visit at study day 0, second visit at study day 30 and third regular visit at day 90 or after drop-out between day 30 and 90. All patients who had at least two study visits (day 0, and between day 0 and day 30) were included in the analysis of variables at day 30. All patients who had a third study visit between day 30 and day 90 (all patients including regular termination and drop-out) were considered for the analysis of variables at day 90 (intention-to-treat analysis).

‡P values of each variable shown at baseline are for individual comparisons of variables. P values of variables of disease activity, and outcomes between different treatment groups after 1 and 3 months, are based on absolute changes from baseline within each treatment group.

3 months, the mean difference in the DAS between the two groups was 0.89 units on a scale from 0 to 10 (4.4% decrease in the MTX group *vs* 16.5% increase in the COL II group with respect to their baseline values), and is clearly higher than the minimal required change of 0.6. In contrast, the patient-assessed RADAI was not different between the two groups.

After 3 months, the physical functional status (HAQ), a patient-oriented disease outcome measure, also showed a significant difference in change from their baseline values between the two groups (0.36 units on a scale from 0 to 3). Absolute and relative differences concerning the health-related quality of life (SF-36, physical component score) between the two groups were 7.4 units on a scale of 100 units with respect to their change from baseline values. All variables of disease activity and short-term disease outcomes in the treatment group receiving MTX were constant over time and did not show any significant changes during the study.

After 3 months of treatment, very similar and highly significant differences in changes in other variables of disease activity (morning stiffness, $P = 0.0031$; CRP, $P = 0.0004$), symptom severity (pain, $P = 0.0005$), functional impairment (muscle strength index, $P = 0.014$) and of an overall assessment of the treat-

TABLE IIb
Table of significance between parameters of disease activity of 75 patients* with rheumatoid arthritis treated with methotrexate or COL II stratified according to disease duration

	Methotrexate *vs* COL II	
Variables	Disease duration <5 yr† MTX, $n = 16$; COL II, $n = 10$	Disease duration $\geqslant 5$ yr MTX, $n = 27$; COL II, $n = 22$
Swollen joints (0–28) (after 3 months)	$P = 0.01$‡	$P < 0.0001$
Tender joints (0–28) (after 3 months)	$P = 0.63$	$P = 0.31$
Erythrocyte sedimentation rate (mm/1 h) (after 3 months)	$P = 0.54$	$P < 0.0001$

*After a study duration of 3 months, only 75 of the initial 92 patients were available for evaluation (see Table IIa).

†Stratification of patients according to disease duration was performed post hoc.

‡P values were calculated using the Mann–Whitney U-test.

TABLE III
Reasons for discontinuation of study therapy in 33 patients with rheumatoid arthritis*

	Methotrexate No. (%)	COL II No. (%)
Total discontinuations†	7 (15.2)	26 (56.5)
Lack of efficacy	6 (13)	24 (52.2)
Adverse reaction	0	0
Other‡	1 (2.2)	2 (4.4)

*For definitions of discontinuation, see Patients and methods.

†For P value of discontinuations between different treatment groups, see Fig. 1.

‡Lack of compliance (two patients) and protocol violation (one patient).

ment at the end of the study by patients ($P < 0.0001$) and the physician ($P < 0.0001$) could be shown between the treatment groups receiving MTX and the group receiving COL II (data not shown). Moreover, patients under MTX did not increase their dosage of NSAIDs and paracetamol as escape medication more frequently than collagen patients (data not shown). On the contrary, between study weeks 3 and 6, patients in the MTX groups used significantly less paracetamol than patients in the COL II group.

Case conclusion (discontinuation or successful termination of the study)

Overall, 33 patients (35.9%) discontinued the study (Table III). The remaining 59 patients completed the study successfully. The significant differences in treatment-related disease activity and disease outcome are reflected by the Kaplan–Meier curve, showing highly significant differences between the number of successful completers (85% successful completers in the group receiving MTX *vs* 43% in the group receiving COL II, $P < 0.0001$) (Fig. 1).

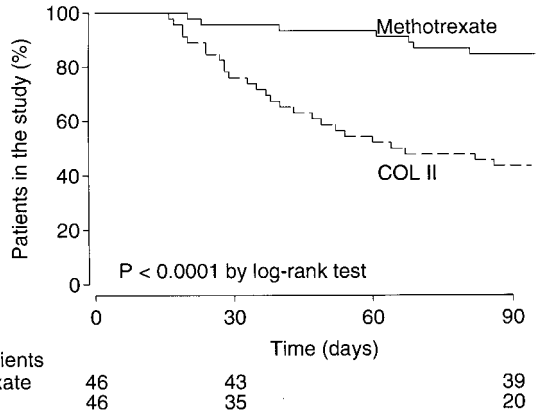

FIG. 1.—Kaplan–Meier curve showing highly significant differences between the number of successful completers (patients remaining in the study for 90 days according to their assigned treatment) in the two treatment groups. There are 85% successful completers in the group receiving methotrexate (39/46) compared with 43% in the group receiving collagen type II (20/46) ($P < 0.0001$). The numbers of patients actively participating at the three study visits (day 0, day 30 and 90) per treatment group are shown below the graph. For details of statistical evaluation, see Patients and methods.

Influence of disease duration on disease activity and successful conclusion of study in patients treated with COL II

In order to attempt to identify patients with a good response to COL II therapy, we performed a post hoc subanalysis of patients with a disease duration of <5 yr (Table IIb). Interestingly, in this subgroup of patients, COL II seemed to sustain better an MTX-induced therapeutic effect on disease activity. In contrast to the statistically significant difference between all patients of the two treatment groups (Table IIa), no or only weak statistically significant differences between COL II- and MTX-treated patients after 3 months could be demonstrated in patients with a disease duration of <5 yr with respect to disease activity. Moreover, in this subgroup, not only 94% of MTX patients, but also 94% of the patients receiving COL II, concluded the 90 study days successfully, compared to only 32% of COL II patients with a disease duration of $\geqslant 5$ yr.

Study subanalysis of patients with a disease duration of <2 yr was not possible due to the very small sample size of only six out of 92 patients.

Adverse events

There were no significant differences in the frequency of adverse events between the two treatment groups, as shown in Table IV. The same was true for the duration and intensity of adverse events (data not shown). Adverse events included nausea and augmented loss of hair in patients on MTX, and heartburn and slight stomach ache in patients with COL II. No serious adverse events were recorded during the entire trial.

TABLE IV
Adverse events related to study medication in patients with rheumatoid arthritis during 3 months of therapy according to their treatment group*

	Methotrexate	COL II	P‡
No. of patients			
Second visit†	45	44	
Third visit	43	32	
Frequency of adverse events (%)			
Second visit	20.0	34.1	0.13
Third visit	30.2	12.5	0.07

*± values are means ± S.D. No serious adverse events were registered during the study.

†Second visit at study day 30 and third regular visit at study day 90 or after drop-out between day 30 and 90.

‡Differences between the two groups were calculated using the χ^2 test.

DISCUSSION

This randomized controlled trial over 3 months in 92 patients with active, long-standing RA demonstrates a highly significant increase in disease activity and significant deterioration of short-term disease outcomes under a treatment with 0.5 mg daily of orally administered COL II from chicken as compared to a continuously given unchanged therapy with MTX. Although the absolute changes in the parameters of disease activity and outcome are not dramatic, the difference in their relative change from baseline was between 60 and 15%, and therefore in part highly significant. The differences in the mean changes in certain parameters from baseline between the two groups were clearly higher than the assumption made to determine the primary endpoint (30% difference in swollen joint count between the two groups) in order to power the study and determine sample size.

A significantly larger number of patients discontinued the study in the collagen treatment group as compared to the MTX group due to a lack of sustaining the former MTX-induced anti-inflammatory effect. Since an increase in dose of NSAIDs to maximal recommended doses and an additional maximal dose of paracetamol up to 4 g was allowed as escape medication, it is reassuring to know that patients on MTX did not increase their dose of these two medications more frequently than collagen patients.

While patient characteristics, pre-existing DMARDs, preparation and dose of COL II between the study of Trentham *et al.* [10] and our study were almost identical, two important differences are worth noting: first, intra-articular corticosteroids were not allowed as escape medication during our study and, second, our collagen dose was 0.5 mg as compared to 0.1 mg used by Trentham *et al.* during the first month. Both factors might, in part, explain the differences in efficacy and premature drop-outs between COL II-treated patients in the two studies. The disease of our patients receiving COL II, regarding the average number of swollen and tender joints (Table IIa), was probably more active compared to the patients in the study by Trentham *et al.* and possibly, therefore, more resistant to COL II treatment after stopping MTX.

In the light of the latest published results of the multicentre, dose-finding, double-blind and placebo-controlled study by Trentham's group, which demonstrates the highest efficacy of orally given COL II in patients with RA at a dose of 20 μg/day [35], our chosen dose of 0.5 mg/day might have been too high. It seems unlikely that MTX, due to the lack of a wash-out period, perturbed the process of oral tolerance with resulting inefficacy of orally given COL II. Weiner's group (H. L Weiner, personal communication) has seen an enhanced oral tolerization effect in RA patients who were on MTX. However, an inhibition of the effect of oral COL II, induced by the withdrawal of MTX in our patients at the beginning of the study (possibly a shift of T cells into a Th1 direction), cannot be excluded.

Although it does not come as a great surprise that patients randomized to COL II did worse than patients remaining on MTX, our comparative study could clearly demonstrate the limitations of COL II in patients with long-standing RA under stable therapy with MTX. However, due to the design and the average disease duration of our patients with RA, we do not provide data on the effect of oral COL II in comparison to placebo in early RA.

We did not follow up all patients systematically, but the patients who were controlled by the out-patient clinic at the department of rheumatology of the University Hospital regained all their previous benefit from MTX after recommencing this therapy.

Sieper *et al.* [11] suggested in their study an identification of patients with a good response to COL II therapy. The groups in that publication had a much shorter disease duration, namely <3 yr. A post hoc analysis of our data revealed the interesting finding that in patients with a disease duration of <5 yr, COL II could sustain an MTX-induced therapeutic effect on disease activity better than in patients with a longer disease duration. In addition, 94% of the patients with a shorter disease duration completed the study successfully as compared with only 32% who had a disease duration of $\geqslant 5$ yr. Owing to the smaller number of patients in this subgroup analysis and the post hoc stratification, a log rank test was not performed between the two groups with different disease duration.

Several questions remain to be answered concerning the therapeutic concept of oral tolerization. What are the optimal doses of orally administered COL II? Which patients with autoimmune diseases, if any, profit most from it? Ongoing studies and future trials have to address these questions in order to clarify further the potential role and effectiveness of COL II as a toleragen in RA.

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